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(54) Title: METHOD AND COMPOSITION TO ENHANCE THE EFFICACY OF A VACCINE USING CHEMOKINES			
(57) Abstract			
<p>The present invention relates to a method to enhance the efficacy of a vaccine in a subject treated with the vaccine comprising administering to the subject in combination with the vaccine a one or more chemokines. The present invention also relates to compositions of vaccines containing chemokines.</p>			

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## METHOD AND COMPOSITION TO ENHANCE THE EFFICACY OF A VACCINE USING CHEMOKINES

### 1. CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 60/069,281 filed December 11, 1997.

### 2. BACKGROUND OF THE INVENTION

The present invention relates to a method to enhance the efficacy of a vaccine by administration of a chemokine, such as macrophage derived chemokine (MDC), in conjunction with the vaccine. The present invention also relates to compositions useful in the method.

#### 2.1. GENERATION OF AN IMMUNE RESPONSE

The introduction of a foreign antigen into an individual elicits an immune response consisting of two major components, the cellular and humoral immune responses, mediated by two functionally distinct populations of lymphocytes known as T and B cells, respectively (see generally Coutinho, 1991, Immune System, *Encyclopedia of Human Biology*, Vol. 4, Ed. Dulbecco, Academic Press, Inc.). A subset of T cells responds to antigen stimulation by producing lymphokines which "help" or activate various other cell types in the immune system.

Another T cell subset is capable of developing into antigen-specific cytotoxic effector cells, which can directly kill antigen-positive target cells. On the other hand, the B cell response is primarily carried out by secretory proteins, antibodies, which directly bind and neutralize antigens.

Helper T cells (TH) can be distinguished from classical cytotoxic T lymphocytes (CTL) and B cells by their cell surface expression of the glycoprotein marker CD4. Although the mechanism by which CD4<sup>+</sup> TH function has not been fully elucidated, the existence of functionally distinct subsets within the CD4<sup>+</sup> T cell compartment has been reported (Mosmann and Coffman, 1989, *Ann. Rev. Immunol.*

7:145-173). In the mouse, type 1 helper T cells (TH1) produce interleukin-2 (IL-2) and  $\tau$ -interferon ( $\tau$ -IFN) upon activation, while type 2 helper T cells (TH2) produce IL-4 and IL-5. Based on the profile of lymphokine production, TH1 appear to be involved in promoting the activation and proliferation of other T cell subsets including CTL, whereas TH2 specifically regulate B cell proliferation and differentiation, antibody synthesis, and antibody class switching.

A second T cell subpopulation is the classical CTL which express the CD8 surface marker. Unlike most TH, these cells display cytolytic activity upon direct contact with target cells, rather than through the production of lymphokines. *In vivo*, CTL function is particularly important in situations where an antibody response alone is inadequate. Significant experimental evidence indicates that CTL rather than B cells and their antibody products play a principal role in the defense against viral infections and cancer.

A salient feature of both T and B cell responses is their exquisite specificity for the immunizing antigen; however, the mechanisms for antigen recognition differ between these two cell types. B cells recognize antigens by antibodies, either acting as cell surface receptors or as secreted proteins, which bind directly to antigens on a solid surface or in solution, whereas T cells only recognize antigens that have been processed or degraded into small fragments and presented on a solid phase such as the surface of antigen-presenting cells (APC). Additionally, antigenic fragments must be presented to T cells in association with major histocompatibility complex (MHC)-encoded class I or class II molecules. The MHC refers to a cluster of genes that encode proteins with diverse immunological functions. In man, the MHC is known as HLA. Class I gene products are found on all somatic cells, and they were originally discovered as targets of major transplantation rejection responses. Class II gene products are mostly expressed on cells of various hematopoietic lineages, and they are involved in cell-cell interactions in the immune system. Most importantly, MHC-encoded proteins have been shown to function as receptors for processed antigenic fragments on the surface of APC (Bjorkman et al., 1987, *Nature* 329:506-512).

Another level of complexity in the interaction between a T cell and an antigenic fragment is that it occurs only if the MHC molecules involved are the same on the APC and the responding T cells. In other words, a T cell specific for a particular antigenic epitope expresses a receptor having low affinity for self MHC



proteins, which when such MHC proteins on APC are occupied by the epitope, engage the T cell in a stronger interaction leading to antigen-specific T cell activation. The phenomenon of a T cell reacting with a processed antigen only when presented by cells expressing a matching MHC is known as MHC-restriction.

The specificity of T cell immune responses for antigens is a function of the unique receptors expressed by these cells. The T cell receptor (TCR) is structurally homologous to an antibody; it is a heterodimer composed of disulfide-linked glycoproteins. Four TCR polypeptide chains known as  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  have been identified, although the vast majority of functional T cells express the  $\alpha\beta$  heterodimeric TCR. Transfer of  $\alpha$  and  $\beta$  genes alone into recipient cells was shown to be both necessary and sufficient to confer antigen specificity and MHC-restriction (Dembic et al., 1986, *Nature* 320:232-238). Thus, the  $\alpha\beta$  TCR appears to be responsible for recognizing a combination of antigenic fragment and MHC determinants.

The apparent basis of MHC restriction is that  $CD4^+$  T cells express  $\alpha\beta$  TCR which recognize antigenic fragments physically associated with MHC class II proteins, while the TCR on  $CD8^+$  CTL recognize MHC class I-associated fragments. Thus,  $CD4^+$  T cells can recognize only a restricted class of APC that are class II<sup>+</sup>, whereas  $CD8^+$  CTL can interact with virtually any antigen-positive cells, since all cells express class I molecules.  $CD4^+$  CTL have been identified, and they are MHC class II restricted, and lyse target cells only if the latter express self-MHC class II determinants associated with specific antigenic fragments. Both CD4 and CD8 molecules also contribute to this interaction by binding to monotypic determinants on the MHC class II and I molecules, respectively.

A second type of TCR composed of  $\gamma\delta$  heterodimers is expressed by a small percentage of T cells, but the involvement of  $\gamma\delta$  T cells in antigen-specific recognition is still poorly understood. Some studies have shown that functionally active  $\gamma\delta$  T cells can be cytolytic in a MHC non-restricted manner.

In summary, the generation of an immune response begins with the sensitization of  $CD4^+$  and  $CD8^+$  T cell subsets through their interaction with APC that express MHC-class I or class II molecules associated with antigenic fragments. The sensitized or primed  $CD4^+$  T cells produce lymphokines that participate in the activation of B cells as well as various T cell subsets. The sensitized  $CD8^+$  T cells increase in numbers in response to lymphokines and are capable of destroying any

cells that express the specific antigenic fragments associated with matching MHC-encoded class I molecules. For example, in the course of a viral infection, CTL eradicate virally-infected cells, thereby limiting the progression of virus spread and disease development.

## 2.2. ANTIGEN PRESENTING CELLS

The presentation of antigens to T cells is carried out by specialized cell populations referred to as antigen presenting cells (APC). Typically, APC include macrophages/monocytes, B cells, and bone marrow-derived dendritic cells (DC). APC are capable of internalizing exogenous antigens, cleaving them into smaller fragments in enzyme-rich vesicles, and coupling the fragments to MHC-encoded products for expression on the cell surface (Goldberg and Rock, 1992, *Nature* 357:375-379). Since APC express both MHC-encoded class I and class II glycoproteins, they can present antigenic fragments to both CD4<sup>+</sup> and CD8<sup>+</sup> T cells for the initiation of an immune response.

By definition, APC not only can present antigens to T cells with antigen-specific receptors, but can provide all the signals necessary for T cell activation. Such signals are incompletely defined, but probably involve a variety of cell surface molecules as well as cytokines or growth factors. Further, the factors necessary for the activation of naive or unprimed T cells may be different from those required for the re-activation of previously primed memory T cells. The ability of APC to both present antigens and deliver signals for T cell activation is commonly referred to as an accessory cell function. Although monocytes and B cells have been shown to be competent APC, their antigen presenting capacities *in vitro* appear to be limited to the re-activation of previously sensitized T cells. Hence, they are not capable of directly activating functionally naive or unprimed T cell populations.

Although it had been known for a long time that APC process and present antigens to T cells, it was not shown until relatively recently that small antigenic peptides could directly bind to MHC-encoded molecules (Babbitt et al., 1985, *Nature* 317:359; Townsend et al., 1986, *Cell* 44:959). However, it is believed that, normally, complex antigens are proteolytically processed into fragments inside the APC, and become physically associated with the MHC-encoded proteins intracellularly prior to

trafficking to the cell surface as complexes. Two distinct pathways for antigen presentation have been proposed (Braciale et al., 1987, *Immunol. Rev.* 98:95-114). It was thought that exogenous antigens were taken up by APC, processed and presented by the exogenous pathway to class II restricted CD4<sup>+</sup> T cells, while the endogenous pathway processed intracellularly synthesized proteins, such as products of viral genes in virally-infected cells, for association with MHC class I proteins and presentation to CD8<sup>+</sup> CTL. However, although the two pathways in antigen processing and presentation may still be correct in some respects, the distinction is blurred in light of recent findings that exogenously added antigens may also be presented to class I-restricted CTL (Moore et al., 1988, *Cell* 54:777).

The term "dendritic cells" (DC) refers to a diverse population of morphologically similar cell types found in a variety of lymphoid and non-lymphoid tissues (Steinman, 1991, *Ann. Rev. Immunol.* 9:271-296). These cells include lymphoid DC of the spleen, Langerhans cells of the epidermis, and veiled cells in the blood circulation. Although they are collectively classified as a group based on their morphology, high levels of surface MHC-class II expression, and absence of certain other surface markers expressed on T cells, B cells, monocytes, and natural killer cells, it is presently not known whether they derive from a common precursor or can all function as APC in the same manner. Further, since the vast majority of published reports have utilized DC isolated from the mouse spleen, results from these studies may not necessarily correlate with the function of DC obtained from other tissue types. (Inaba et al., 1997, *J. Exp. Med.* 166:182-194; Hengel et al., 1987, *J. Immunol.*, 139:4196-4202; Kaut et al., 1988, *J. Immunol.*, 140:3186-3193; Romani et al., 1989, *J. Exp. Med.* 169:1169-1178; Macatonia et al., 1989, *J. Exp. Med.* 169:1255-1264; Inaba et al., 1990, *J. Exp. Med.* 172:631-6640). For example, despite high levels of MHC-class II expression, mouse epidermal Langerhans cells, unlike splenic DC, are not active APC in mixed leucocyte reaction (MLR), unless cultured with granulocyte-macrophage colony stimulating factor (GM-CSF) (Witmer-Pock et al., 1987, *J. Exp. Med.* 166:1484-1498; Heufler et al., 1988, *J. Exp. Med.* 167:700-705). Most human Langerhans cells express the CD1 and CD4 markers, while blood DC do not. Additionally, it has not been established the extent to which the functional characteristics observed with mouse DC are applicable to human DC, especially the DC obtained from non-splenic tissues; in part, due to inherent differences between the

human and murine immune systems.

Recently, a few studies have described the isolation of human DC from the peripheral blood, which involves the use of sheep red blood cells and/or fetal calf serum (Young and Steinman, 1990, *J. Exp. Med.* 171:1315-1332; Freudenthal and Steinman, 1990, *Proc. Natl. Acad. Sci. USA* 87:7698-7702; Macatonia et al., 1989 *Immunol.* 67:285-289; Markowicz and Engleman, 1990, *J. Clin. Invest.* 85:955-961). Engleman et al. described a partial purification procedure of DC from human blood, which does not involve the use of sheep red blood cells and/or fetal calf serum, and showed that the partially purified human DC can, in fact, present exogenous antigens to naive T cells (PCT Publication WO 94/02156 dated February 3, 1994 at page 9, lines 5-32).

Recent studies have indicated that DCs are superior APCs as compared to other APCs such as macrophages and monocytes. First, the potent accessory cell function of DCs provides for an antigen presentation system for virtually any antigenic epitopes which T and B cells are capable of recognizing through their specific receptors. For example, Engleman et al. demonstrate that human DCs can present both complex protein antigens and small peptides to CD4<sup>+</sup> T cells as well as to CD8<sup>+</sup> CTL (PCT Publication WO 94/02156 dated February 3, 1994, Example 7, from page 29, line 10 to page 34, line 16). Engleman et al. also show that the *in vitro* priming effect of DCs does not require the addition of exogenous lymphokines, indicating that DCs produce all of the necessary signals in antigen presentation leading to the activation of T cells (PCT Publication WO 94/02156 dated February 3, 1994, from page 32, line 36 to page 33, line 2). More importantly, DCs can induce a primary CD4<sup>+</sup> T cell-mediated proliferative response when similarly prepared monocytes can not induce such a response (PCT Publication WO 94/02156 dated February 3, 1994 at page 31, lines 23-30). Similarly, when DCs and monocytes were compared for their ability to present antigens for re-activating secondary T cell response, it was observed that DCs were capable of stimulating a stronger response than monocytes (PCT Publication WO 94/02156 dated February 3, 1994 at page 32, lines 12-16).

### 2.3. CHEMOKINES

Chemokines, or chemoattractant cytokines, are a subgroup of immune factors

that have been shown to mediate chemotactic and other pro-inflammatory phenomena (see, Schall, 1991, *Cytokine* 3:165-183). Chemokines are small molecules of approximately 70-80 residues in length and can generally be divided into two subgroups,  $\alpha$  which have two N-terminal cysteines separated by a single amino acid (CxC) and  $\beta$  which have two adjacent cysteines at the N terminus (CC). RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$  are members of the  $\beta$  subgroup (reviewed by Horuk, R., 1994, *Trends Pharmacol. Sci.* 15:159-165; Murphy, P.M., 1994, *Annu. Rev. Immunol.* 12:593-633; Baggiolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

MCP-1 has been shown to attract monocytes but not neutrophils. MCP-1, MCP-2, and MCP-3 share a pyroglutamate proline NH<sub>2</sub>-terminal motif and are structurally closely related to each other and to eotaxin (56% to 71% amino acid sequence identity). MCP-1, MCP-2, and MCP-3 attract monocytes, CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes (Loetscher et al. *FAESB J.* 1994, 8:1055-60), as well as basophil leukocytes. MCP-2, MCP-3, and MCP-4 (but not MCP-1) attracts eosinophil leukocytes. All four MCPs attract activated T lymphocytes, natural killer (NK) cells, and dendritic cells (see Baggiolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

Eotaxin acts on eosinophils and is inactive on neutrophils and monocytes, but has weak-to-moderate chemotactic activity toward IL-2-conditioned T lymphocytes (see Baggiolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705). Due to its preferential, powerful action on eosinophils and its occurrence in different species, eotaxin is considered to be an important chemokine in the pathophysiology of allergic conditions and asthma (See Baggiolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

IP10 is a CXC chemokine attracts human monocytes, T lymphocytes, and NK cells, and Mig attracts tumor-infiltrating T lymphocytes. It has been suggested that IP10 and Mig may also be involved in the regulation of lymphocyte recruitment and the formation of the lymphoid infiltrates observed in autoimmune inflammatory lesions, delayed-type hypersensitivity, some viral infections, and certain tumors (Baggiolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

SDF-1 (stromal cell-derived factor 1), including SDF-1 and SDF-1 $\beta$  stimulates the proliferation of B cell progenitors, and attracts mature dendritic cells (Finkel et al. *Immunobiology* 1998, 198:490-500). Synthetic human SDF-1 stimulates monocytes, neutrophils, and peripheral blood lymphocytes, as is indicated by [Ca<sup>2+</sup>]<sub>i</sub> changes and chemotaxis. SDF-1 is also a powerful HIV-suppressive factor (See Baggiolini et al.

*Annu. Rev. Immunol.* 1997, 15:675-705).

The amino terminus of the  $\beta$  chemokines RANTES, MCP-1, and MCP-3 has been implicated in the mediation of cell migration and inflammation induced by these chemokines. This involvement is suggested by the observation that the deletion of the amino terminal 8 residues of MCP-1, amino terminal 9 residues of MCP-3, and amino terminal 8 residues of RANTES and the addition of a methionine to the amino terminus of RANTES, antagonize the chemotaxis, calcium mobilization and/or enzyme release stimulated by their native counterparts (Gong et al., 1996, *J. Biol. Chem.* 271:10521-10527; Proudfoot et al., 1996 *J. Biol. Chem.* 271:2599-2603). Additionally,  $\alpha$  chemokine-like chemotactic activity has been introduced into MCP-1 via a double mutation of Tyr 28 and Arg 30 to leucine and valine, respectively, indicating that internal regions of this protein also play a role in regulating chemotactic activity (Beall et al., 1992, *J. Biol. Chem.* 267:3455-3459).

The monomeric forms of all chemokines characterized thus far share significant structural homology, although the quaternary structures of  $\alpha$  and  $\beta$  groups are distinct. While the monomeric structures of the  $\beta$  and  $\alpha$  chemokines are very similar, the dimeric structures of the two groups are completely different. An additional chemokine, lymphotactin, which has only one N terminal cysteine has also been identified and may represent an additional subgroup ( $\gamma$ ) of chemokines (Yoshida et al., 1995, *FEBS Lett.* 360:155-159; and Kelner et al., 1994, *Science* 266:1395-1399).

Receptors for chemokines belong to the large family of G-protein coupled, 7 transmembrane domain receptors (GCR's) (See, reviews by Horuk, R., 1994, *Trends Pharmacol. Sci.* 15:159-165; and Murphy, P.M., 1994, *Annu. Rev. Immunol.* 12:593-633). Competition binding and cross-desensitization studies have shown that chemokine receptors exhibit considerable promiscuity in ligand binding. Examples demonstrating the promiscuity among  $\beta$  chemokine receptors include: CCR-1, which binds RANTES and MIP-1 $\alpha$  (Neote et al., 1993, *Cell* 72:415-425), CCR-4, which binds RANTES, MIP-1 $\alpha$ , and MCP-1 (Power et al., 1995, *J. Biol. Chem.* 270:19495-19500), and CCR-5, which binds RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$  (Alkhatib et al., 1996, *Science* 272:1955-1958 and Dragic et al., 1996, *Nature* 381:667-674). Erythrocytes possess a receptor (known as the Duffy antigen) which binds both  $\alpha$  and  $\beta$  chemokines (Horuk et al., 1994, *J. Biol. Chem.* 269:17730-17733; Neote et al., 1994, *Blood* 84:44-52; and Neote et al., 1993, *J. Biol. Chem.* 268:12247-12249). Thus the sequence and

structural homologies evident among chemokines and their receptors allow some overlap in receptor-ligand interactions.

Godiska et al. identified and described the nucleic acid and amino acid sequences of an additional  $\beta$  chemokine designated macrophage derived chemokine (MDC) (PCT Publication WO 96/40923 dated December 19, 1996, and 1997, *J. Exp. Med.* 185:1595-1604). PCT publication WO 96/40923 further provides materials and methods for the recombinant production of the chemokine, the purified and isolated chemokine protein, and polypeptide analogues thereof. The PCT publication WO 96/40923 does not disclose that the human MDC has chemotactic activity upon DC. While Godiska et al. (1997, *J. Exp. Med.* 185:1595-1604) showed that, in a microchamber migration assay, monocyte-derived DC migrated toward the human MDC, the reference fails to teach that MDC can enhance an immune response to an antigen *in vivo*.

Chang et al. (1997, *J. Biol. Chem.* 272(40):25229-25237), isolated a stimulated T cell chemotactic protein (STCP-1) from an activated macrophage cDNA library. The nucleotide sequence of the STCP-1 is identical to that of the MDC isolated by Godiska et al. (PCT Publication WO 96/40923 dated December 19, 1996, and 1997, *J. Exp. Med.* 185:1595-1604). However, unlike the results observed by Godiska et al. (1997, *J. Exp. Med.* 185:1595-1604), Chang et al. (1997, *J. Biol. Chem.* 272(40):25229-25237) showed that although the STCP-1 acted as a mild chemoattractant for primary activated T lymphocytes and a potent chemoattractant for chronically activated T lymphocytes, the STCP-1 has no chemoattractant activity for monocytes, neutrophils, eosinophils and resting T lymphocytes. Chang et al. further showed that the STCP-1 does not induce  $\text{Ca}^{2+}$  mobilization in monocytes, dendritic cells, neutrophils, eosinophils, lipopolysaccharide-activated B lymphocytes, and freshly isolated resting T lymphocytes.

## 2.4. HIV VACCINES

Human immunodeficiency virus (HIV) induces a persistent and progressive infection leading, in the vast majority of cases, to the development of the acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983, *Science* 220:868-870; Gallo et al., 1984, *Science* 224:500-503). The HIV envelope surface glycoproteins are

synthesized as a single 160 kilodalton precursor protein which is cleaved by a cellular protease during viral budding into two glycoproteins, gp41 and gp120. gp41 is a transmembrane glycoprotein and gp120 is an extracellular glycoprotein which remains non-covalently associated with gp41, possibly in a trimeric or multimeric form (Hammerskjold, M. and Rekosh, D., 1989, *Biochem. Biophys. Acta* 989:269-280). The V3 loop of gp120 is the major determinant of sensitivity to chemokine inhibition of infection or replication (Cocchi et al., 1996, *Nature Medicine* 2:1244-1247; and Oravec et al., 1996, *J. Immunol.* 157:1329-1332).

Although considerable effort is being put into the design of effective therapeutics, currently no curative anti-retroviral drugs against AIDS exist. The HIV-1 envelope proteins (gp160, gp120, gp41) have been shown to be the major antigens for neutralizing anti-HIV antibodies present in AIDS patients (Barin et al., 1985, *Science* 228:1094-1096). Thus far, therefore, these proteins seem to be the most promising candidates to act as antigens for anti-HIV vaccine development. Several groups have begun to use various portions of gp160, gp120, and/or gp41 as immunogenic targets for the host immune system (see, for example, Ivanoff et al., U.S. Pat. No. 5,141,867; Saith et al., PCT publication WO 92/22654; Shafferman, A., PCT publication WO 91/09872; Formoso et al., PCT publication WO 90/07119). Therefore, methods to increase the efficacy of vaccines against HIV, especially vaccines using gp120 as the antigen, are needed.

Additionally a novel vaccine technology, designated genetic vaccination, nucleic acid vaccination or DNA vaccination, has been explored to induce immune responses *in vivo*. Injection of cDNA expression cassettes results in *in vivo* expression of the encoded proteins (Dubensky et al., 1984, *Proc. Natl. Acad. Sci. USA* 81:7529-7533; Raz et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4523; Wolff et al., 1990, *Science* 247:1465-1468), with the concomitant development of specific cellular and humoral immune responses directed against the encoded antigen(s) (Wang et al., 1995, *Hum. Gene Ther.* 6:407-418; Ulmer et al., 1993, *Science* 259:1745-1749; Tang et al., 1992, *Nature* 356:152-154; Michel et al., 1995, *Proc. Natl. Acad. Sci. USA* 92:5307-5311; and Lowrie et al., 1994, *Vaccine* 12:1537-1540). Humoral and cellular responses have been induced to HIV-1 and SIV antigens through various applications of this technology in macaques (Wang et al., 1995, *Virology* 221:102-112; Wang et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4156-4160; and Boyer et al., 1996, *J. Med.*



*Primatol.* 25:242-250) as well as mice (Wang et al., 1995, *Virology* 221:102-112; Lu et al., 1995, *Virology* 209:147-154; Haynes et al., 1994, *AIDS Res. Hum. Retroviruses* 10 (Suppl. 2):S43-S45; Okuda et al., 1995, *AIDS Res. Hum. Retroviruses* 11:933-943).

Recently, Lekutis et al. (1997, *J. Immunol.* 158:4471-4477), assessed the TH cell response elicited by an HIV-1 gp120 DNA vaccine in rhesus monkeys by isolation of gp120-specific, MHC class II-restricted CD4<sup>+</sup> T cell lines from the vaccinated animals. Lekutis et al. showed that the isolated cell lines proliferated in response to APC in the presence of recombinant gp120, as well as to APC expressing HIV encoded env protein. Lekutis et al. further showed that these cell lines responded to env by secreting IFN- $\gamma$  and IFN- $\alpha$  without appreciable IL-4 production. These results demonstrate that the animals exhibited a cellular immune response to the DNA vaccine.

Boyer et al. (1997, *Nature Medicine* 3:625-532), inoculated chimpanzees with an HIV-1 DNA vaccine encoding env, rev, and gag/pol, and found that the immunized animals developed specific cellular and humoral immune responses to these proteins. After challenging the immunized animals with a heterologous chimpanzee titrated stock of HIV-1 SF2, Boyer et al. further found, using a Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay, that those animals vaccinated with the DNA vaccine were protected against infection whereas the control animals were not so protected.

Kim et al., (1997 *J. Immunol.* 158:816-826), investigated the role of co-delivery of genes for IL-12 and GM-CSF along with DNA vaccine formulation for HIV-1 antigens env and gag/pol in mice. Kim et al. observed a dramatic increase in specific CTL response from the mice immunized with the HIV-1 DNA vaccine and IL-12. Kim et al. also observed that the co-delivery of IL-12 genes resulted in the reduction of specific antibody response, whereas the codelivery of GM-CSF genes resulted in the enhancement of specific antibody response. Kim et al. further observed that co-delivery of IL-12 gene with a HIV DNA vaccine results in splenomegaly (Kim et al. 1997, *J. Immunol.*, 158:816-826), which has been shown in mice to have toxic effects such as weight reduction or even death (Eng et al., 1995, *J. Exp. Med.* 181:1893; Stevensen et al., 1995, *J. Immunol.* 155:2545; and Orange et al., 1995, *J. Exp. Med.* 181:901).

Notwithstanding the recent developments of the HIV DNA vaccine, there still

exists a need for a method to enhance the efficacy of a vaccine, especially an HIV DNA vaccine. For instance, for efficacious vaccine against HIV-1 one preferably induces both cellular and humoral immune responses to control the infection (Boyer et al., 1997, *Nature Medicine* 3:625-532). The induction of both cellular and humoral immune response by the Berjer et al. method is still quite low because only one of the three immunized chimpanzees developed both cellular and humoral responses. Similarly, although co-delivery of an IL-12 encoding gene with a HIV DNA vaccine, as described in Kim et al. (1997, *J. Immun.* 158:816-826), may have enhanced the cellular immune response, this co-delivery also decreased the humoral response.

Citation of a reference hereinabove shall not be construed as an admission that such reference is prior art to the present invention.

### 3. SUMMARY OF THE INVENTION. SUMMARY OF THE INVENTION. . SUMMARY OF THE INVENTION

The present invention is based upon the ability of chemokines, such as MDC, Rantes, MIP-1d, MIP-1B, and I-309, to enhance the immune response to an antigen, particularly a vaccine. Accordingly, in a first aspect, the present invention provides a method for enhancing the efficacy of a vaccine, which method comprises administration to a subject of one or more purified chemokines, or biologically active fragments, analogues or derivatives thereof, either concurrently with one or more purified antigens against which an immune response is desired or within a time period either before or after administration of the antigens such that the immune response against the antigens is enhanced.

In a second aspect, the present invention provides a method to enhance the efficacy of a vaccine, which method comprises administration to a subject of a first set of one or more purified nucleic acids comprising one or more nucleotide sequences encoding one or more chemokines, or fragments, derivatives, analogues, and/or truncation isoforms thereof, and a second purified nucleic acid comprising a nucleotide sequence encoding one or more antigens against which an immune response is desired, such that, the one or more chemokine(s) and the antigen(s) are expressed in a coordinated manner upon introduction into a suitable cell. Alternatively, the nucleotide sequences encoding one or more chemokines, or

fragments, derivatives, and/or analogues thereof, and the antigens against which an immune response is desired are present on the same nucleic acid.

In a preferred embodiment, the invention provides a method to enhance the efficacy of an HIV vaccine.

In yet another aspect, the present invention provides a composition comprising an immunogenic amount of one or more purified antigens, an amount of one or more purified chemokines, or a fragments, derivatives, analogues and/or truncation isoforms thereof, effective to enhance the immune response to the antigen. In another aspect, the present invention provides a composition comprising a first set of one or more purified nucleic acids comprising one or more nucleotide sequences encoding one or more chemokines, fragments, derivatives analogues and or truncation isoforms thereof, and a second set of purified nucleic acids comprising one or more nucleotide sequences encoding one or more antigens against which an immune response is desired, such that, the chemokine(s) and the antigen are expressed in a coordinated manner upon introduction into a suitable cell. In a preferred embodiment, the antigen is an HIV antigen. In another preferred embodiment, the chemokine is selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EB11-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha

chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

#### 4. DESCRIPTION OF FIGURES

Figures 1A and 1B. The nucleotide and amino acid sequences of MDC. 1A depicts the nucleotide sequence of MDC (SEQ ID NO:1), with the coding region indicated by the appearance of the amino acid sequence in the line below; and 1B depicts the amino acid of MDC (SEQ ID NO:2) from GenBank accession no. U83171 (Godiska et al., 1997, *J. Exp. Med.* 185:1595-1604).

#### 5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for enhancing the efficacy of a vaccine in a subject comprising administering to the subject one or more purified antigens in conjunction with one or more purified chemokines, or more purified fragments, derivatives or analogues and/or truncation isoforms thereof.

While any chemokine may be employed according to the present invention, the chemokine is preferably selected from the following table:

Chemokine Class	Chemokines	Abbreviations	Accession Number
CC Chemokines	Macrophage-derived chemokine	MDC/STCP-1	u83171; u83239
	Monocyte chemotactic protein 1	MCP-1	x14768
	Monocyte chemotactic protein 2	MCP-2	X99886
	Monocyte chemotactic protein 3	MCP-3	x72308; s57464
	Monocyte chemotactic protein 4	MCP-4	u46767
	activated macrophage specific chemokine 1	AMAC-1	Y13710
	Macrophage inflammatory protein 1 alpha	MIP-1 $\alpha$	AF043339; X03754; D90144

Chemokine Class	Chemokines	Abbreviations	Accession Number
CC Chemokines (continued)	Macrophage inflammatory protein 1 beta	MIP-1 $\beta$	j04130; d90145
	Macrophage inflammatory protein 1 gamma	MIP-1 $\gamma$	
	Macrophage inflammatory protein 1 delta	MIP-1 $\delta$	AF031587
	Macrophage inflammatory protein 2 alpha	MIP-2 $\alpha$	AF043340
	Macrophage inflammatory protein 3 alpha	MIP-3 $\alpha$	u77035
	Macrophage inflammatory protein 3 beta	MIP-3 $\beta$	u77180
	Regulated upon activation, normal T cell expressed and secreted (and its variants)	RANTES	M21211
	I-309		M57502
	EBI1-ligand chemokine	ELC	AB000887
	Pulmonary and activation regulated chemokine	PARC/DC-CK-1/MIP4	AB000221
	Liver and activation-regulated chemokine	LARC	D86955
	Thymus and activation regulated chemokine	TARC	D43767
	Eotaxin (and variants)		D49372; Z69291; Z75669; Z75668
	Human chemokine 1	HCC1; NCC2	Z49270; z49269
	Human chemokine 2	HCC2; NCC3, MIP-5, MIP-1 $\delta$	Z70292
	Human chemokine 3	HCC3	Z70293
	IL-10-inducible chemokine	HCC4	U91746
	liver-expressed chemokine	LEC; HCC4; NCC4	AB007454
	6Ckine		AF001979
	Exodus 1		u64197
	Exodus 2		U88320
	Exodus 3		U88321
	thymus-expressed chemokine	TECK	U86358
	Secondary Lymphoid tissue chemokine	SLC	AB002409

Chemokine Class	Chemokines	Abbreviations	Accession Number
CC Chemokines (continued)	Lymphocyte and Monocyte chemoattractant; Monotactin	LMC	AF055467
	Activation-induced, chemokine-related molecule	ATAC	x86474
	Myeloid progenitor inhibitory factor-1	MPIF-1; MIP-3 or ckbeta8	u85767
	Myeloid progenitor inhibitory factor-2	MPIF-2	u85768
	Stromal cell-derived factor 1 alpha	SDF-1 $\alpha$ ; PBSF	L36034
CXC chemokines	Stromal cell-derived factor 1 beta	SDF-1 $\beta$ ; PBSF	L36033
	B-cell-attracting chemokine 1	BLC	AJ002211
	HuMIG		x72755 s60728
	H174		AF002985
	Interferon-stimulated T-cell alpha chemoattractant	ITAC	AF030514
	Interleukin-8	IL-8	m17017; y00787
	IP-10		X02530
	platelet factor 4	PF4	M20901
	growth-regulated gene-alpha	GRO- $\alpha$	J03561
	growth-regulated gene-beta	GRO- $\beta$	M36820
	growth-regulated gene-gamma	GRO- $\gamma$	M36821
	Neutrophil-activating protein 2	NAP-2; CTAP-3	M54995; M38441
	ENA-78		L37036
	granulocyte chemotactic protein 2	GCP-2	Y08770
C-CHEMOKINES	LYMPHOTACTIN	SCM-1	D63789 D63790
CX3C-CHEMOKINES	Fractalkine/neurotactin		U91835 U84487

The present invention also relates to the use of fragments, analogues and derivatives of the foregoing chemokines, as well as truncation isoforms of such chemokines which are known in the art.

The present invention also relates to therapeutic compositions comprising one or more chemokines, nucleic acids encoding one or more chemokines, derivatives, analogues, and/or truncation isoforms thereof, and nucleic acids encoding the same, that are effective to enhance the immune response of a subject to a vaccine.

In another preferred embodiment of the invention, nucleic acids comprising

nucleotide sequences encoding one or more chemokines or fragments or derivatives, including truncation isoforms, thereof, and encoding one or more antigens against which an immune response is desired, which coding sequences are operatively linked to gene regulatory sequences capable of directing the expression of the one or more chemokines and the one or more antigens upon introduction into a suitable cell, for example, but not limited to, the cell (of a subject), are administered to a subject such that the one or more chemokines, or fragments or derivatives, including truncation isoforms, thereof, and one or more antigens, are expressed in the subject.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

#### **5.1. METHODS AND COMPOSITIONS TO ENHANCE THE EFFICACY OF A VACCINE**

The present invention provides methods for enhancing the efficacy of a vaccine in a subject, which methods comprise administering to a subject an immunogenic amount of one or more purified antigens against which an immune response is desired in the subject in conjunction with an amount of one or more purified chemokines, or fragments, derivatives, analogues and/or truncation isoforms thereof, effective to enhance the immune response against the antigen. In one aspect, the purified chemokine(s), or fragment(s), derivative(s), analogue(s) and/or truncation isoforms thereof, are administered to the subject concurrently with (e.g., in the same composition with) the purified antigen or antigens against which an immune response is desired. In another, aspect, the purified chemokine(s), or fragment(s), derivative(s), analogue(s) and/or truncation isoforms thereof, are administered either before or after the administration of one or more purified antigens against which immunity is desired in the subject, but is administered within such time that the chemokine(s) enhance the immune response to the one or more antigens. For example, but not by way of limitation, the purified chemokine(s) are administered during the time that the subject mounts an immune response against the administered one or more antigens, or, the purified MDC is administered within, for example, but not limited to, 30 minutes, 1 hour, 5 hours, 10 hours, 1 day, 2 days of (preferably, after) administration of the one or more purified antigens against which immunity is desired.

In a preferred embodiment, the present invention provides compositions comprising an immunogenic amount of one or more purified antigens and an amount of purified MDC, or one or more fragments, derivatives or analogues thereof, effective to enhance the immune response to said antigen and, preferably, the composition further comprises a pharmaceutically acceptable carrier.

A preferred chemokine for use in the methods and compositions of the present invention is any MDC protein, fragment or derivative thereof, that is capable of enhancing the efficacy of a vaccine (for example, but not limited to, as determined by the assays described in Section 5.4, *infra*). In one specific embodiment, the MDC is purified full length MDC, preferably full length MDC having the amino acid sequence of SEQ ID NO: 2 (Figure 1B). In another embodiment, the MDC is a purified protein, the amino acid sequence of which consists of amino acid numbers 2-69 of SEQ ID NO: 2 (Figure 1B). In another specific embodiment, the MDC is a purified protein, the amino acid sequence of which consists of amino acid numbers 3-69 of SEQ ID NO: 2 (Figure 1B). In still another specific embodiment, the MDC is a purified protein, the N-terminal amino acid sequence of which consists of the amino acid sequence Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg-Asp-Tyr-Val-Arg-Tyr-Arg-Leu (portion of SEQ ID NO: 2). In yet another specific embodiment, the MDC is a purified protein, the N-terminal amino acid sequence of which consists of the amino acid sequence Pro-Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg (portion of SEQ ID NO: 2). In yet another specific embodiment, the MDC is a purified derivative of a protein, the N-terminal amino acid sequence of which protein consists of the amino acid sequence Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg-Asp-Tyr-Val-Arg-Tyr-Arg-Leu (SEQ ID NO: 2), which derivative has activity to enhance the efficacy of the vaccine. In yet another specific embodiment, the MDC is a purified derivative of a protein, the N-terminal amino acid sequence of which protein consists of the amino acid sequence Pro-Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg (SEQ ID NO: 2), which derivative has activity to enhance the efficacy of the vaccine.

In yet another specific embodiment, the chemokine is a purified derivative of the protein, which derivative has one or more insertions of or substitutions with one or more non-classical amino acids relative to a corresponding wildtype chemokine, which derivative will enhance the efficacy of the vaccine. In yet another specific



embodiment, the chemokine is a purified derivative of the protein that has only one or more conservative substitutions in sequence relative a corresponding wildtype chemokine, which derivative will enhance the efficacy of the vaccine. The chemokines useful in the present invention may be derived from any suitable source and obtained by any method known in the art, for example but not limited to the methods described in Section 5.2 *infra*.

Preferably, the chemokine(s) are of the same species as the subject to which the vaccine is administered. In a preferred embodiment, one or more human chemokines are administered to a human subject, e.g., human MDC is administered to a human subject, alone or in combination with another chemokine.

The present invention also provides a method to enhance the efficacy of a vaccine in a subject, which method comprises administering to a subject a purified first nucleic acid comprising a nucleotide sequence encoding an antigen against which an immune response is desired in a subject and a purified second nucleic acid comprising a nucleotide sequence encoding one or more chemokines, or fragment(s), derivative(s) or analogue(s) thereof, where the expression of the encoded antigen(s) and chemokine(s), or fragment(s), derivative(s) or analogue(s) thereof, are under control of one or more appropriate gene regulatory elements (which regulatory elements can be any regulatory element known in the art, for example, but not limited to, those regulatory elements described in Section 5.2 *supra*), such that, upon introduction of said first and second nucleic acids into a suitable cell (e.g., a cell of the subject), the antigen and chemokine(s), or fragment(s), derivative(s) or analogue(s) thereof, are coordinately expressed, *i.e.*, are expressed either at the same time or within an appropriate time period (*i.e.*, sufficient for the chemokine(s) to enhance the immune response against the antigen relative to a corresponding immune response in the absence of the chemokine) and the antigen(s) are expressed in an immunogenic amount and the chemokine(s), or fragment(s), derivative(s) or analogue(s) thereof, are expressed in an amount sufficient to enhance the immune response against the antigen(s). In a specific embodiment, the nucleotide sequences encoding the chemokine(s) and the antigen are present on separate nucleic acids. In another embodiment, the nucleotide sequences encoding the chemokine(s) and the antigen(s) are present on the same nucleic acid.

The present invention also provides compositions to enhance the

efficacy of a vaccine in a subject, which compositions comprise a purified first nucleic acid comprising a nucleotide sequence encoding one or more antigen(s) and a purified second nucleic acid comprising a nucleotide sequence encoding one or more chemokines, or fragments or derivatives, including truncation isoforms, thereof, wherein the nucleotide sequences encoding the antigens and the chemokine(s) are operably linked to one or more gene regulatory elements such that, upon introduction of said first and second nucleic acids into a suitable cell (e.g., a cell of the subject), the antigen(s) and chemokine(s) are expressed in a coordinated manner and the antigen(s) are expressed in an immunogenic amount and the chemokine(s) are expressed in an amount effective to enhance the immune response against the antigen, relative to a corresponding immune response in the absence of such chemokine(s).

The present invention also provides compositions to enhance the efficacy of a vaccine in a subject, which compositions comprise a purified first set of one or more purified nucleic acids comprising one or more nucleotide sequences encoding one or more antigens and a purified second set of one or more purified nucleic acids comprising a nucleotide sequence encoding one or more chemokines, or fragments, analogues, derivatives, (including truncation isoforms) thereof, wherein the nucleotide sequence(s) encoding the antigen(s) and the chemokine(s) are operably linked to one or more gene regulatory elements such that, upon introduction of said first and second sets of nucleic acids into a suitable cell (e.g., a cell of the subject), the antigen(s) and chemokine(s) are expressed in a coordinated manner and the antigen(s) are expressed in an immunogenic amount and the chemokine(s) are expressed in an amount effective to enhance the immune response against the antigen, relative to a corresponding immune response in the absence of such chemokine(s).

The present invention also provides compositions to enhance the efficacy of a vaccine in a subject, which compositions comprise a purified nucleic acid comprising a first set of one or more nucleotide sequences encoding one or more antigens and a second set of one or more nucleotide sequence encoding one or more chemokines, or fragments, derivatives, or analogues thereof (including truncation isoforms), wherein the first and second sets of nucleotide sequences are operably linked to one or more gene regulatory elements such that, upon introduction into a suitable cell, the antigen(s) and the chemokine(s) are expressed in a coordinated manner and the antigen(s) are expressed in an immunogenic amount and the chemokine(s) are

expressed in an amount effective to enhance the immune response against the antigen(s).

Any nucleic acid comprising a nucleotide sequence encoding one or more chemokine proteins, or fragments or derivatives, thereof (including truncation isoforms), that are capable of enhancing the immune response to the antigen (for example, but not limited to, as determined by any of the assays described in Section 5.2., *infra*) can be used in the methods and compositions of the present invention.

In a preferred embodiment, the nucleotide sequence encodes MDC. In another embodiment, the MDC-encoding nucleotide consists of the nucleotide sequence of SEQ ID NO:1 (Figure 1A). In another specific embodiment, the method or composition of the invention uses a nucleic acid encoding an MDC derivative having deletional, insertional or substitutional mutations and combination thereof, which derivative has activity to enhance the immune response against an antigen in a subject.

Such compositions of nucleic acids encoding an antigen are often referred to as DNA vaccines.

Such DNA vaccines are produced by any method known in the art for constructing an expression plasmid vector containing the nucleotide sequences of the antigen(s) and/or chemokine(s) to be expressed which vector is suitable for expression of the encoded proteins in the subject or in cells recombinant for the expression vector, which cells are to be provided to the subject. Such expression vectors may contain various promoters, terminators and polyadenylation coding regions to control the expression of the encoded protein.

The DNA vaccine can be administered by any method known in the art for administration of DNA. The DNA vaccine may be delivered either directly, in which case the subject is directly exposed to the DNA vaccine such that the DNA enters and is expressed in cells of the subject, or indirectly, in which case, the DNA vaccine is first introduced into suitable cells by any method known in the art *in vitro*, then the cells containing the DNA vaccine are transplanted into the subject.

In a specific embodiment, the DNA vaccine is directly administered *in vivo*, where it is expressed to produce the encoded antigens and chemokine(s). This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or

other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic; Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In a preferred embodiment, the nucleic acid of a DNA vaccine is injected into the muscle of the subject to be immunized.

Another approach is to introduce the nucleic acid of the DNA vaccine into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign nucleic acid into cells (see e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92 (1985)) and may be used in accordance with the present invention. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene.

Cells into which a DNA vaccine can be introduced for purposes of immunization encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

The resulting recombinant cells can be delivered to a subject by various

methods known in the art. In a preferred embodiment, the recombinant cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The cells can also be encapsulated in a suitable vehicle and then implanted in the subject (see, e.g., Dionne et al. PCT Publication WO 92/19195, dated November 12, 1992). The amount of cells envisioned for use depends on the desired effect, subject state, etc., and can be determined by one skilled in the art.

By way of example, and not by way of limitation a DNA vaccine may be generated as described by Lekutis et al. for an HIV DNA vaccine (1997, *J. Immunol.* 158:4471-4477). Briefly, an expression vector is constructed with the promoter, enhancer and intron A of human cytomegalovirus (CMV) and the termination and polyadenylation sequences of bovine growth hormone in a plasmid backbone. Additionally, the nucleotide sequence for signal sequence of tissue plasminogen activator is either substituted for the signal sequence of the antigen, if the antigen has a signal sequence or is added onto the amino-terminus of the antigen, thereby eliminating the dependence on viral proteins for expression (e.g., in the case of gp120 expression, rev and env proteins are required unless the HIV-1 signal sequence is so substituted). The resulting formulation is then injected intra-muscularly.

Further examples of DNA vaccines are set forth in Boyer et al. (1996, *J. Med. Primatol.*, 25:242-250), which describes the construction of a plasmid encoding the HIV-1 gp160 envelope glycoprotein as well as the rev-tax region cloned into pMAMneoBlue vector (Clontech, Inc., Palo Alto, CA), and a vector encoding the envelope glycoprotein and rev from HIV-1 strain MN under the control of the CMV promoter. Another vector which can be used in the present invention is as described in Boyer et al. (1997, *Nature Medicine* 3:526-532) and contains expression cassettes encoding the envelope and Rev proteins of HIV-1 strain MN, and encoding the Gag/Pol proteins of HIV-1 strain IIIB.

For the practice of the present invention, the nucleotide sequence for the one or more chemokines, or fragments, derivatives, or analogues thereof, can either be incorporated into the same expression vector containing the nucleotide sequence encoding the antigen in such a manner that the chemokine(s) are expressed. Alternatively, the nucleotide sequence encoding the chemokine(s), or fragment(s),

derivative(s) or analogue(s) thereof, can be cloned into a separate expression vector (e.g., as described above for the expression vector containing the sequences coding for antigen) and the expression vector that expresses the antigen(s) mixed with the expression vector that expresses the chemokine(s). The mixture of the two expression vectors can then be administered to the subject.

The methods and compositions of the present invention may be used as a vaccine in a subject in which immunity for the antigen(s) is desired. Such antigens can be any antigen known in the art to be useful in a vaccine formulation. The methods and compositions of the present invention can be used to enhance the efficacy of any vaccine known in the art. The vaccine of the present invention may be used to enhance an immune response to infectious agents and diseased or abnormal cells, such as but not limited to bacteria, parasites, fungi, viruses, tumors and cancers. The compositions of the invention may be used to either treat or prevent a disease or disorder amenable to treatment or prevention by generating an immune response to the antigen provided in the composition. In one preferred embodiment, the antigen(s) are proteins, fragments or derivatives, including truncation isoforms, thereof, encoded by any genes of the HIV genome including the *env*, *gag*, *pol*, *nef*, *vif*, *rev*, and *tat* genes. In a more preferred embodiment, the antigen is an HIV-associated gp120 protein.

The methods and compositions of the present invention may be used to elicit a humoral and/or a cell-mediated response against the antigen(s) of the vaccine in a subject. In one specific embodiment, the methods and compositions elicit a humoral response against the administered antigen in a subject. In another specific embodiment, the methods and compositions elicit a cell-mediated response against the administered antigen in a subject. In a preferred embodiment, the methods and compositions elicit both a humoral and a cell-mediated response.

The subjects to which the present invention is applicable may be any mammalian or vertebrate species, which include, but are not limited to, cows, horses, sheep, pigs, fowl (e.g., chickens), goats, cats, dogs, hamsters, mice and rats, monkeys, rabbits, chimpanzees, and humans. In a preferred embodiment, the subject is a human. The compositions and methods of the invention can be used to either prevent a disease or disorder, or to treat a particular disease or disorder, where an immune response against a particular antigen or antigens is effective to treat or prevent the

disease or disorder. Such diseases and disorders include, but are not limited to, viral infections, such as HIV, CMV, hepatitis, herpes virus, measles, etc, bacterial infections, fungal and parasitic infections, cancers, and any other disease or disorder amenable to treatment or prevention by eliciting an immune response against a particular antigen or antigens. In another preferred embodiment, the subject is infected or at risk of being infected with HIV virus.

In another preferred embodiment the invention provides methods and compositions to enhance the efficacy of an HIV vaccine, such a vaccine can be administered to either prevent or treat HIV.

## 5.2. CHEMOKINE GENES AND PROTEINS

Chemokine proteins and nucleic acids can be obtained by any method known in the art. Chemokine nucleotide and amino acid sequences are available in public databases such as Genbank and are also published in various references known to those of skill in the art. The gene bank accession numbers for the preferred chemokines of the present invention are provided in Table I, in Section 5 above. The ensuing discussion uses MDC by way of example, but applies equally to other chemokines as well.

The MDC nucleotide and amino acid sequences for, *inter alia*, human, are available in the public databases (e.g. Genbank accession No. U83171) also published in Godiska et al., 1997, *J. Exp. Med.* 185:1595-1604. The nucleotide sequence and the amino acid sequence for the human MDC are provided in Figures 1A and B (SEQ ID NOS:1 and 2, respectively).

Chemokines used herein include, but are not limited to, chemokines from mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees, and human. In one preferred embodiment, the chemokine is of human origin.

Any vertebrate cell potentially can serve as the nucleic acid source for the isolation of chemokine nucleic acids. The nucleic acid sequences encoding the chemokine(s) can be isolated from vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, as well as additional primate sources, etc. The DNA may be obtained by standard procedures known in the art from cloned DNA (e.g., a

DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see, for example, Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from cDNA, cDNA is generated from totally cellular RNA or mRNA by methods that are well known in the art. The gene may also be obtained from genomic DNA, where DNA fragments are generated (e.g. using restriction enzymes or by mechanical shearing), some of which will encode the desired gene. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing all or a portion of the chemokine gene may be accomplished in a number of ways.

A preferred method for isolating a chemokine gene is by the polymerase chain reaction (PCR), which can be used to amplify the desired chemokine sequence in a genomic or cDNA library or from genomic DNA or cDNA that has not been incorporated into a library. Oligonucleotide primers which would hybridize to chemokine sequences can be used as primers in PCR.

Additionally, a portion of the chemokine (of any species) gene or its specific RNA, or a fragment thereof, can be purified (or an oligonucleotide synthesized) and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, *Science* 196:180; Grunstein, M. And Hogness, D., 1975, *Proc. Natl. Acad. Sci. U.S.A.* 72:3961). Those DNA fragments with substantial homology to the probe will hybridize. Chemokine nucleic acids can be also identified and isolated by expression cloning using, for example, anti-chemokine antibodies for selection.

Alternatives to obtaining the chemokine DNA by cloning or amplification



include, but are not limited to, chemically synthesizing the gene sequence itself from the known chemokine sequence or making cDNA to the mRNA which encodes the chemokine protein. Other methods are possible and within the scope of the invention. Once a clone has been obtained, its identity can be confirmed by nucleic acid sequencing (by any method well known in the art) and comparison to known chemokine sequences. DNA sequence analysis can be performed by any techniques known in the art, including but not limited to the method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger, F., et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699), use of an automated DNA sequenator (e.g., Applied Biosystems, Foster City, CA) or the method described in PCT Publication WO 97/ 15690.

Nucleic acids which are hybridizable to a chemokine nucleic acid; or to a nucleic acid encoding a chemokine derivative can be isolated, by nucleic acid hybridization under conditions of low, high, or moderate stringency (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792). For example, the nucleic acid of SEQ ID No: 1 is hybridizable to an MDC nucleic acid.

Chemokine proteins and derivatives, analogs and fragments of chemokine proteins can be obtained by any method known in the art, including but not limited to recombinant expression methods, purification from natural sources, and chemical synthesis.

For example, chemokines can be obtained by recombinant protein expression techniques. For recombinant expression, the chemokine gene or portion thereof is inserted into an appropriate cloning vector for expression in a particular host cell. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site

desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and chemokine gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated chemokine gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequence coding for a chemokine protein or a functionally active analog or fragment or other derivative thereof, can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native chemokine gene and/or its flanking regions. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein

coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a chemokine protein or peptide fragment may be regulated by a second nucleic acid sequence so that the chemokine protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a chemokine protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control chemokine expression include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the  $\beta$ -lactamase promoter (Villa-Komaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region

which is active in myeloid cells (Mogham et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

For example, a vector can be used that comprises a promoter operably linked to a chemokine-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (e.g., an antibiotic resistance gene).

In a specific embodiment, an expression construct is made by subcloning a chemokine coding sequence into the *EcoRI* restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; Smith and Johnson, 1988, Gene 7:31-40). This allows for the expression of the chemokine protein product from the subclone in the correct reading frame.

Expression vectors containing chemokine gene inserts can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a chemokine gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted chemokine gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a chemokine gene in the vector. For example, if the chemokine gene is inserted within the marker gene sequence of the vector, recombinants containing the chemokine insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the chemokine protein in *in vitro* assay systems, e.g., binding with anti-chemokine antibody or the chemokine's receptor.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host

system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous protein. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

In other specific embodiments, the chemokine protein(s), fragment(s), analogue(s), or derivative(s) may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. In a specific embodiment, a chimeric protein containing all or a portion of the chemokine is joined via a peptide bond to all or a portion of an antigen against which immunity is desired.

Both cDNA and genomic sequences can be cloned and expressed.

The chemokine protein(s) may also be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column

chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay (see Section 5.5). Alternatively, the protein can be synthesized by standard chemical methods known in the art (e.g., see Hunkapiller, M., et al., 1984, *Nature* 310:105-111). The chemokine-encoding nucleic acid sequence(s) can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions. Any technique for mutagenesis known in the art can be used, including, but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551), use of TAB linkers (Pharmacia), mutation-containing PCR primers, etc.

The experimentation involved in mutagenesis consists primarily of site-directed mutagenesis followed by phenotypic testing of the altered gene product. Some of the more commonly employed site-directed mutagenesis protocols take advantage of vectors that can provide single stranded as well as double stranded DNA, as needed. Generally, the mutagenesis protocol with such vectors is as follows. A mutagenic primer, i.e., a primer complementary to the sequence to be changed, but consisting of one or a small number of altered, added, or deleted bases, is synthesized. The primer is extended *in vitro* by a DNA polymerase and, after some additional manipulations, the now double-stranded DNA is transfected into bacterial cells. Next, by a variety of methods, the desired mutated DNA is identified, and the desired protein is purified from clones containing the mutated sequence. For longer sequences, additional cloning steps are often required because long inserts (longer than 2 kilobases) are unstable in those vectors. Protocols are known to those skilled in the art and kits for site-directed mutagenesis are widely available from biotechnology supply companies, for example from Amersham Life Science, Inc. (Arlington Heights, IL) and Stratagene Cloning Systems (La Jolla, CA).

In other specific embodiments, the chemokine derivative(s) or analogue(s) may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analogue, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art.

In addition, chemokine proteins, derivatives (including fragments and chimeric proteins), and analogues can be chemically synthesized. See, e.g., Clark-Lewis et al., 1991, *Biochem.* 30:3128-3135 and Merrifield, 1963, *J. Amer. Chem. Soc.* 85:2149-2156. For example, chemokines, derivatives and analogues can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 50-60). Chemokines, derivatives and analogues that are proteins can also be synthesized by use of a peptide synthesizer. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49).

The chemokine proteins, derivatives, or analogues of the invention may be synthesized in their entirety by the sequential addition of amino acid residues or alternatively as fragment subcomponents which may be combined using techniques well known in the art, such as, for example, fragment condensation (Shin et al., 1992, *Biosci. Biotech. Biochem.* 56:404-408; Nyfeler et al., 1992, *Peptides*, Proc. 12th Amer. Pep. Soc., Smith and Rivier (eds), Leiden, pp 661-663); and Nokihara et al., 1990, *Protein Research Foundation*, Yanaihara (ed), Osaka, pp 315-320).

In a less preferred embodiment, chemokine derivatives can be obtained by proteolysis of the protein followed by purification using standard methods such as those described above (e.g., immunoaffinity purification).

In another alternate embodiment, native chemokine proteins can be purified from natural sources, by standard methods such as those described above (e.g., immunoaffinity purification).

### 5.3. COMPOSITION FORMULATIONS AND METHODS OF ADMINISTRATION

The composition formulations of the invention comprise an effective immunizing amount of an immunologically active ingredient, i.e., one or more antigens, and an amount of one or more chemokine(s), or fragment(s) or derivative thereof, effective to enhance the immune response against the antigen in a subject, and a pharmaceutically acceptable carrier or excipient. In a specific embodiment, the

chemokines are selected from the group consisting of Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EB11-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

Pharmaceutically acceptable carriers or excipients are well known in the art and include but are not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, sterile isotonic aqueous buffer, and combinations thereof. One example of such an acceptable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc. The carrier is preferably sterile. The formulation should suit the mode of administration.

In addition, if desired, the vaccine or composition preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine or composition. Suitable adjuvants may include, but are not limited to: mineral gels,



e.g., aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols; polyanions; peptides; oil emulsions; alum, MDP, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine. The effectiveness of an adjuvant may be determined by comparing the induction of antibodies directed against a MDC-containing composition in the presence and in the absence of various adjuvants.

In instances where the recombinant antigen is a hapten, i.e., a molecule that is antigenic in that it can react selectively with cognate antibodies, but not immunogenic in that it cannot elicit an immune response, the hapten may be covalently bound to a carrier or immunogenic molecule; for instance, a large protein such as serum albumin will confer immunogenicity to the hapten coupled to it. The hapten-carrier may be formulated for use as a vaccine.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

The chemokine(s), or fragment(s) or derivative(s) thereof, and/or the antigen(s) may be formulated into the composition as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids, such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with free carboxyl groups may also be derived from inorganic bases, such as, for example, sodium potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

The vaccines of the invention may be multivalent or univalent. Multivalent vaccines are made from recombinant viruses that direct the expression of more than one antigen.

An effective dose (immunizing amount) is that amount sufficient to produce an immune response to the antigen(s) in the host to which the vaccine preparation is administered. The precise dose of the composition to be employed in the formulation will depend on the route of administration, and the nature of the subject to be

immunized, and should be decided by the practitioner according to standard clinical techniques. Effective doses of the vaccines or compositions of the present invention may also be extrapolated from dose-response curves derived from animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers comprising one or more of the ingredients of the composition formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, a lyophilized immunologically active ingredient and one or more chemokine polypeptide(s) of the invention are provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (e.g., 0.005% brilliant green).

Many methods may be used to introduce the composition formulations of the invention; these include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, e.g., using a bifurcated needle).

The DNA vaccines of the invention can be administered by any method known in the art for delivery of DNA to subject (for example, as described in Section 5.3 *supra*)

#### 5.4. DETERMINATION OF COMPOSITION EFFICACY

The activity of one or more chemokines, or a fragment, derivative or analogue thereof, to enhance immune response to an antigen can be determined by monitoring the immune response in test animals following immunization with a composition containing the chemokine(s) and an antigen and comparing the response to that following immunization with the antigen in the absence of the chemokine(s). Generation of a humoral (antibody) response and/or cell-mediated immunity, may be taken as an indication of an immune response. Test animals may include mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees, etc., and eventually human subjects. Assays for humoral and cell-mediated immunity are well known in the art.

Methods of introducing the composition may include oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal or any other standard routes of immunization. The immune response of the test subjects can be analyzed by various approaches well known in the art, such as but not limited to: testing the reactivity of the resultant immune serum to the antigen of the chemokine-containing vaccine, as assayed by known techniques, e.g., immunosorbant assay (ELISA), immunoblots, radioimmunoprecipitations, etc.

As one example of suitable animal testing, a composition of the present invention may be tested in mice for the ability to enhance an antibody response to an antigen (using for example, but not limited to, the method as described in Section 6, *infra*) and the delayed-type hypersensitivity (DTH) response (also described in Section 6 *infra*), measured by an increase in footpad swelling after inoculation in the footpad of the test animal, as compared to the measurements in animals administered the antigen in a composition not containing chemokine. For example, as test animals BALB/c mice may be used. The test group each receives an inoculation with fixed amount of antigen and varying amount of one or more chemokines. The control group receives an inoculation of comparable amount of antigen alone.

Serum samples may be drawn from the mice after the final inoculation (for example every one or two weeks after inoculation), and serum is analyzed for antibodies against the antigen using known methods in the art, e.g., using an ELISA. DTH responses to the antigen may be measured after the final inoculation (e.g. within 1-7 days). An increase in the serum titer of antibodies recognizing the antigen and/or

an increase in footpad swelling in the animals receiving the antigen-compositions containing the chemokine(s) as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing the chemokine(s), indicates that the chemokine(s) enhance the immune response to antigen. An increase in the serum titer of antibodies recognizing the antigen and/or an increase in footpad swelling in the animals receiving the antigen-compositions containing the chemokines as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing chemokine(s), indicates that the chemokine(s) enhances the immune response to antigen. An increase in the serum titer of antibodies recognizing the antigen and/or an increase in footpad swelling in the animals receiving the antigen-compositions containing MDC as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing MDC, indicates that the MDC enhances the immune response to antigen. An increase in the serum titer of antibodies recognizing the antigen and/or an increase in footpad swelling in the animals receiving the antigen-compositions containing MDC as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing MDC, indicates that the MDC enhances the immune response to antigen.

## **6. EXAMPLE: IMMUNIZATION WITH MDC-CONTAINING COMPOSITION**

The following experiment illustrates the evaluation of whether MDC will act as an adjuvant for a protein antigen and enhance the efficacy of a vaccine. However, it will be appreciated that the description applies equally to other chemokines and combinations of chemokines.

### **6.1. MATERIALS AND METHODS**

#### **6.1.1. ANIMALS AND REAGENTS**

BALB/c mice are purchased from Harlan-Sprague-Dawley (Indianapolis, IN).

Human MDC (hMDC) was obtained from CD8<sup>+</sup> T cell clones immortalized *in vitro* prepared as previously described (Markham et al., 1983 *Int. J. Cancer* 31:413; Markham et al. 1984, *Int. J. Cancer* 33:13). One such immortalized CD8<sup>+</sup> T cell clone, F3b Clone 19, was adapted to growth in serum-free medium by the following procedure and used for further studies. F3b Clone 19 cells were grown in complete medium containing rIL-2 (16 ng/ml) at 37°C in a CO<sub>2</sub> incubator. After expanding the culture to 200 ml, the cells were pelleted and resuspended in RPMI medium containing HB101 (Irvine Scientific) supplemented with 16 ng/ml of rIL-2, 1% glutamine and 1% penicillin/streptomycin. The cells were grown to full confluence and the medium harvested by centrifugation at 670 x g for 10 minutes.

Human MDC (hMDC) was purified from F3b Clone 19 as described in Pal et al., 1997, *Science* 278:695-698. Briefly, the cell free culture supernatant from F3b Clone 19 was clarified by high speed centrifugation and fractionated by heparin affinity chromatography, taking advantage of the heparin binding characteristics of chemokines (Witt and Lander, 1994, *Current Biology* 4:394; Proost et al., 1996, *Method: A Companion to Methods in Enzymology* 10:82). Culture supernatant (1200 ml) from F3b Clone 19, grown to high cell density in serum-free medium supplemented with rIL-2 was clarified by high speed centrifugation (100,000 x g for 60 minutes at 4°C) and applied to a 5 ml HiTrap heparin affinity FPLC column (Pharmacia) equilibrated in 10 mM Tris-HCl, pH 7.6 containing 0.1 M NaCl (column buffer). The column was then washed extensively with column buffer and the bound proteins eluted from the column with 10 mM Tris-HCl, pH 7.6 containing 2.0 M NaCl at a flow rate of 0.5 to 1 ml/minute. Virtually all of the HIV suppressive activity effective against primary NSI and SI isolates and HIV-1<sub>1118</sub> was recovered in the column eluate (data not shown). The heparin affinity column eluate was brought to pH 2.0 by addition of trifluoroacetic acid (TFA) and subjected to reversed phase HPLC on a PEEK C-18 column (Waters Instruments) equilibrated in H<sub>2</sub>O containing 0.1 % TFA. Proteins bound to the column were eluted with a 5 minute linear gradient of aqueous acetonitrile (0 to 35 %) containing 0.1% TFA. After 10 minutes at 35% acetonitrile, the column was further developed with a 60 minute linear gradient of 35-70% aqueous acetonitrile in TFA. The flow rate was maintained at 0.5 to 1 ml/minute. The fractions obtained were then tested for suppressor activity in the acute infectivity assay using HIV-1<sub>1118</sub>. Active fractions were pooled, diluted twofold in H<sub>2</sub>O with 0.1 % TFA

and reapplied to the column. The column was then developed with a 30 minute linear aqueous acetonitrile gradient (0-60%) containing 0.1% TFA at a flow rate of 0.5 to 1 ml/minute. The fractions obtained were assayed as above. Active fractions were pooled, diluted with H<sub>2</sub>O/0.1 % TFA and fractionated under the same conditions to obtain a single protein peak. The fraction corresponding to the peak and flanking fractions were tested in the infectivity assay to verify that suppressor activity was cofractionated with the protein.

Suppressive activity against HIV-1<sub>IIIB</sub> in the absence of cytotoxic effects consistently copurified with a single protein peak that appeared as a homogeneous 8 kDa band when analyzed by SDS-polyacrylamide gel electrophoresis. This protein was not reactive in ELISAs for RANTES, MIP-1 $\alpha$  or MIP-1 $\beta$  (R&D Systems).

Recombinant gp120 protein derived from HIV-1 IIIB isolate is purchased from Intracel (Foster City, CA).

#### 6.1.2 IMMUNIZATION OF MICE

The hMDC and the gp120 is resuspended in a total volume of 50  $\mu$ l of phosphate-buffered saline (PBS). Mice are divided into 5 groups with 3-4 mice in each group. Groups 1-4 are inoculated with 10  $\mu$ g gp120 and 0.3  $\mu$ g, 0.1  $\mu$ g, 0.03  $\mu$ g, and 0.01  $\mu$ g of hMDC, respectively. As a control, group 5 is inoculated with 10  $\mu$ g of gp120 in the absence of hMDC. For primary inoculation, each group of mice is inoculated with 10  $\mu$ l of the hMDC and gp120 solution via footpad. Two to three weeks after the primary inoculation, each mouse is given the same dose of hMDC/gp120 that is used in primary inoculation.

#### 6.1.3 ELISA ASSAY

Serum samples are collected one week after the second inoculation via tail vein bleed. gp120 serum responses are measured using standard gp120 antibody ELISA assays.

#### 6.1.4 DTH ASSAY

The delayed-type hypersensitivity (DTH) response is measured from 1-7 days after the second inoculation. A caliper is to be used to measure footpad swelling.

## 6.2. RESULTS

Mice inoculated with hMDC/gp120 are expected to have greater serum antibody and DTH responses than mice inoculated with gp120 alone. The improved responses will be reflected in either increased titers of serum antibody responses or increased footpad swelling. A dose response effect is expected - increasing the dose of hMDC used is expected to cause a corresponding improvement in the serum and DHT gp120-specific responses.

## 7. EXAMPLE: OTHER CHEMOKINES AND COMBINATIONS OF CHEMOKINES

The foregoing experiments can be repeated using other chemokines and combinations of chemokines. For example, the experiments are preferably repeated using one or more chemokines selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine., 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.



**THE CLAIMS:**

1. A method to enhance the efficacy of a vaccine in a subject comprising administering to the subject an immunogenic amount of one or more purified antigens against which an immune response is desired in the subject and an amount of one or more chemokines, or purified fragments or derivatives thereof, effective to enhance the efficacy of said vaccine.
2. The method of claim 1, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
3. The method of claim 1, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4; activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-

- regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.
4. The method of claim 1, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.
  5. The method of claim 1 wherein the fragment(s) or derivative(s) are truncation isoforms.
  6. The method of claim 1, wherein the one or more chemokines include MDC comprising the amino acid sequence of SEQ ID NO: 2.
  7. The method of claim 1, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2.
  8. The method of claim 1, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2., which derivative has activity to enhance the efficacy of the vaccine.
  9. The method of claim 1, wherein the one or more chemokine derivatives has one or more insertions or substitutions with one or more non-classical amino acids relative to a corresponding wildtype chemokine, which derivative has activity to enhance the efficacy of the vaccine.
  10. The method of claim 1, including a chemokine derivative having one or more conservative substitutions in sequence relative a wildtype MDC, which derivative has activity to enhance the efficacy of the vaccine.
  11. The method of claim 1, wherein the one or more chemokines include a human chemokine.

12. The method of claim 1, wherein the purified chemokine(s) or purified fragment(s) or derivative(s) thereof is/are administered concurrently with the purified antigen(s).
13. The method of claim 1 wherein the purified chemokine(s) or purified fragment(s) or derivative(s) thereof, are administered within a time period before or after administration of the purified antigen, which time period permits the purified MDC or purified fragment or derivative thereof MDC to enhance the efficacy of the vaccine.
14. The method of claim 1, wherein the antigen is an HIV antigen.
15. The method of claim 14, wherein the HIV antigen is HIV-associated gp120 protein.
16. The method of claim 1, wherein the subject is a human.
17. The method of claim 1, wherein the subject is infected or at risk of being infected with HIV virus.
18. The method of claim 1, wherein the vaccine elicits a humoral response against the antigen in the subject.
19. The method of claim 1, wherein the vaccine elicits a cell-mediated response against the antigen in the subject.
20. The method of claim 1, wherein the vaccine elicits both a humoral and a cell-mediated response against the antigen in the subject.
21. The method of claim 1, wherein the vaccine further comprises pharmaceutically acceptable excipient, auxiliary substance, adjuvant, wetting or emulsifying agent, or pH buffering agent.

22. A method to enhance the efficacy of a vaccine in a subject comprising administering to the subject a first amount of a first set of one or more purified nucleotide sequences encoding one or more antigens against which an immune response is desired in the subject and a second second set of one or more purified nucleic acids, each comprising a nucleotide sequence encoding one or more chemokines, or fragments or derivatives thereof, wherein the antigen(s) and the chemokine(s) are expressed in a coordinated manner upon introduction into a suitable cell, said first amount is immunogenic and said second amount is effective in enhancing the efficacy of the vaccine.
23. The method of claim 22, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
24. The method of claim 22, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EB11-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine

- 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.
25. The method of claim 22, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.
26. The method of claim 22 wherein the fragment(s) or derivative(s) are truncation isoforms.
27. The method of claim 22, wherein the nucleotide sequence encoding one or more chemokines comprises the nucleotide sequence of SEQ ID NO:1.
28. The method of claim 22, wherein one or more of the chemokine derivative(s) have deletional, insertional or substitutional mutations and combination thereof, which derivative has activity to enhance the efficacy of the vaccine.
29. The method of claim 22, wherein the vaccine elicits a humoral response against the antigen in the subject.
30. The method of claim 22, wherein the vaccine elicits a cell-mediated response against the antigen in the subject.
31. The method of claim 22, wherein the vaccine elicits both a humoral and a cell-mediated response against the antigen in the subject.
32. The method of claim 22, wherein the vaccine further comprises pharmaceutically acceptable excipient, auxiliary substance, adjuvant, wetting or emulsifying agent, or pH buffering agent.
33. A composition comprising: an immunogenic amount of one or more purified antigens and an amount of one or more purified chemokines, or purified

- fragments or derivatives thereof, effective to enhance the immune response to said antigen(s); and a pharmaceutically acceptable carrier.
34. The composition of claim 33, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.
  35. The composition of claim 33, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
  36. The composition of claim 33, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating

- protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.
- 37. The composition of claim 33, wherein the fragment(s) or derivative(s) are truncation isoforms.
- 38. The composition of claim 33, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2.
- 39. The composition of claim 33, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2, which derivative has activity to enhance the efficacy of the vaccine.
- 40. The composition of claim 33, wherein the one or more chemokine derivatives has one or more insertions of or substitutions with one or more non-classical amino acids relative to a corresponding wildtype chemokine, which derivative has activity to enhance the efficacy of the vaccine.
- 41. The composition of claim 33, wherein the one or more chemokine derivatives has one or more conservative substitutions in sequence relative a corresponding wildtype chemokine, which derivative has activity to enhance the efficacy of the vaccine.
- 42. The composition of claim 33, wherein the chemokine is a human chemokine.
- 43. The composition of claim 33, wherein the antigen is an HIV antigen.
- 44. The composition of claim 43, wherein the antigen is HIV associated gp120 protein.
- 45. A composition comprising an amount of a first set of purified nucleic acids comprising one or more nucleotide sequences encoding one or more antigens

- and a second set of purified nucleic acids comprising one or more nucleotide sequences encoding one or more chemokines, or fragments or derivatives thereof, wherein the antigen(s) and the chemokine(s), or fragment(s) or derivative(s) thereof, are expressed from said first set of nucleic acid(s) and second set of nucleic acid(s) in a coordinated manner such that upon introduction into a suitable cell, the amount of said first set of nucleic acid(s) is sufficient to express an immunogenic amount of the antigen and the amount of the said second set of nucleic acid(s) is effective in enhancing the efficacy of the vaccine; and a pharmaceutically acceptable carrier.
46. The composition of claim 45, wherein the chemokine is MDC and the nucleic acid encoding the MDC comprises the nucleotide sequence of SEQ ID NO: 1.
47. The composition of claim 45, wherein the chemokine derivative(s) have deletional, insertional or substitutional mutations and/or combinations thereof, and the derivative(s) have activity to enhance the efficacy of the vaccine.
48. The composition of claim 45, further comprising pharmaceutically acceptable excipient, auxiliary substance, adjuvant, wetting or emulsifying agent, or pH buffering agent.
49. A composition comprising a first set of purified nucleotide sequences encoding one or more antigens and a second set of purified nucleotide sequences encoding one or more chemokines, or fragments or derivatives thereof, wherein the antigen(s) and the chemokine(s) are expressed in a coordinated manner such that upon introduction into a suitable cell, the sets produce an amount of said antigen(s) that is immunogenic and an amount of chemokine(s), or fragment(s) or derivative(s) thereof, that is effective in enhancing the efficacy of the vaccine relative to a corresponding vaccine composition without such chemokine(s), fragment(s) or derivative(s) thereof.
50. The composition of claim 49, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine,



Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EB11-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

51. The method of claim 49, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
52. The method of claim 49, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.
53. The composition of claim 49, wherein the fragment(s) or derivative(s) are truncation isoforms.

- 54. The composition of claim 49, wherein the nucleic acid is administered directly to the subject.
- 55. The composition of claim 49, wherein the nucleic acid is introduced into a suitable host cell and said suitable host cell is introduced into the subject.

GAGACATACA GGACAGAGC ATG GCT CGC CTA CAG ACT GCA CTC CTG GTT GTC	52
Met Ala Arg Leu Gln Thr Ala Leu Leu Val Val	-24 -20 -15
CTC GTC CTC CTT GCT GTG GCG CTT CAA GCA ACT GAG GCA GGC CCC TAC	100
Leu Val Leu Leu Ala Val Ala Leu Gln Ala Thr Glu Ala Gly Pro Tyr	-10 -5 1
GGC GCC AAC ATG GAA GAC AGC GTC TGC TGC CGT GAT TAC GTC CGT TAC	148
Gly Ala Asn Met Glu Asp Ser Val Cys Cys Arg Asp Tyr Val Arg Tyr	5 10 15
CGT CTG CCC CTG CGC GTG GTG AAA CAC TTC TAC TGG ACC TCA GAC TCC	196
Arg Leu Pro Leu Arg Val Val Lys His Phe Tyr Trp Thr Ser Asp Ser	20 25 30 35
TGC CCG AGG CCT GGC GTG GTG TTG CTA ACC TTC AGG GAT AAG GAG ATC	244
Cys Pro Arg Pro Gly Val Val Leu Leu Thr Phe Arg Asp Lys Glu Ile	40 45 50
TGT GCC GAT CCC AGA GTG CCC TGG GTG AAG ATG ATT CTC AAT AAG CTG	292
Cys Ala Asp Pro Arg Val Pro Trp Val Lys Met Ile Leu Asn Lys Leu	55 60 65

FIG. 1A-1

AGC CAA TGAAGAGCCT ACTCTGATGA CCGTGGCCTT GGCTCCTCCA GGAAGGCTCA 348  
Ser Gln

GGAGCCCTAC CTCCCCTGCCA TTATAGCTGC TCCCCGCCAG AAGCCTGTGC CAACTCTCTG 408

CATTCCCCTGA TCTCCATCCC TGTGGCTGTC ACCCTTGGTC ACCTCCGTGC TGCTACTGCC 468

ATCTCCCCC TGACCCCTCT AACCCTATCCT CTGCCCTCCCT CCCTGCAGTC AGAGGGTCCT 528

GTTCCCATCA GCGATTCCCC TGCTTAAACC CTTCCATGAC TCCCCACTGC CCTAAGCTGA 588 2/6

GGTCAGTCTC CCAAGCCTGG CATGTGGCCC TCTGGATCTG GGTTCCATCT CTGTCTCCAG 648

CCTGCCCACT TCCCTTCATG AATGTGGGT TCTAGCTCCC TGTTCTCCAA ACCCATACTA 708

CACATCCCAC TTCTGGGTCT TTGCCTGGGA TGTGTGTGAC ACTCAGAAAG TCCCACCACC 768

TGCACATGTG TAGCCCCACC AGCCCTCCAA GGCATTGCTC GCCCAAGCAG CTGGTAATTC 828

CATTTCATGT ATTAGATGC CCCTGGCCCT CTGTCCCCCTC TTAATAACCC TAGTCACAGT 888

CTCCGCAGAT TCTTGGGATT TGGGGGTTT CTCCCCCACC TCTCCACTAG TTGGACCAAG 948

FIG. 1A-2

<u>GTTTCTAGCT AAGTTACTCT AGTCTCCAAG CCTCTAGCAT AGAGCACTGC AGACAGGCC</u>	<u>1008</u>
TGGCTCAGAA TCAGAGCCCA GAAAGTGGCT GCAGACAAA TCAATAAAC TAATGTCCT	1068
CCCCCTCTCCC TGCCAAAAGG CAGTTACATA TCAATACAGA GACTCAAGGT CACTAGAAAT	1128
GGGCCAGCTG GGTCATGTG AAGCCCCAAA TTTGCCCCAGA TTCACCTTTC TTCCCCACT	1188
CCCTTTTTTT TTTTTTTTTT TTTGAGATGG AGTTTCGCTC TTGTCACCCA CGCTGGAGTG	1248
CAATGGTGTG GTCTTGGCTT ATTGAAGCCT CTGCCCTCCTG GGTCAAGTG ATTCTCTTGC	1308
CTCAGCCTCC TGAGTAGCTG GGATTACAGG TTCCTTGCTAC CACGCCCAGC TAAATTTTGT	1368
ATTTTATAGTA GAGACGAGGC TTCACCATGT TGGCCAGGCT GGTCTCGAAC TCCTGTCCTC	1428
AGGTAATCCG CCCACCTCAG CCTCCCAAAG TGCTGGGATT ACAGGCGTGA GCCACAGTGC	1488
CTGGCCTCTT CCCTCTCCCC ACTGCCCCCC CCAACTTTTT TTTTTTTTTT ATGGCAGGGT	1548
CTCACTCTGT CGCCCAGGCT GGAGTGCAGT GCGGTGATCT CGGCTCACTA CAACCTCGAC	1608
CTCCTGGGTT CAAGTGATTC TCCCACCCCA GCCTCCCAAG TAGCTGGGAT TACAGGTGT	1668

**FIG. 1A-3**

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<u>TGCCACTACG GCTGGCTAAT TTTTGTAATTT TTAGTAGAGA CAGGTTTCAC CATATTGGCC</u>	<u>1728</u>
AGGCTGGTCT TGAACCTCCTG ACCTCAAGTG ATCCACCTTC CTTGTGCTCC CAAAGTGCTG	1788
AGATTACAGG CGTGAGCTAT CACACCCAGC CTCCTCCCTTT TTTTCCCTAAT AGGAGACTCC	1848
TGTACCTTTC TTCGTTTTAC CTATGTGTCG TGTCTGCTTA CATTTCCCTTC TCCCTCAGG	1908
CTTTTTTTGG GTGGTCCTCC AACCTCCAAT ACCCAGGCCT GGCTCTCTCA GAGTACCCCC	1968
CATTCCACTT TCCCTGCCTC CTTCCTTAAA TAGCTGACAA TCAAATTCAT GCTATGGTGT	2028
GAAAGACTAC CTTTGACTTG GTATTATAAG CTGGAGTTAT ATATGTATTT GAAAACAGAG	2088
TAAATACTTA AGAGGCCCAA TAGATGAATG GAAGAAATTT AGGAACTGTG AGAGGGGGAC	2148
AAGGTGAAGC TTTCTCTGGC CTGGGAGGAA GCTGGCTGTG GTAGCGTAGC GCTCTCTCTC	2208
TCTGTCTGTG GCAGGAGCCA AAGAGTAGGG TGTAATTGAG TGAAGGAATC CTGGGTAGAG	2268
ACCATCTCTA GGTGGTTGGG CCAGGCTAAA GACTGGGAGT TGGGTCTATC TATGCCTTTC	2328
TGGCTGATTT TTGTAGAGAC GGGGTTTTGC CATGTTACCC AGGCTGGTCT CAAACTCCTG	2388

**FIG. 1A-4**

GGCTCAAGCG ATCCTCCTGG CTCAGCCTCC CAAAGTGCTG GGATTACAGG CGTGAATCAC	2448
TGCGCCTGGC TTCCTCTTCC TCTTGAGAAA TATTCTTTTC ATACAGCAAG TATGGGACAG	2508
CAGTGTCCCA GGTAAGGAC ATAAATGTTA CAAGTGTCTG GTCCTTTCTG AGGAGGCTG	2568
GTGCCGCTCT GCAGGGTATT TGAACCTGTG GAATTGGAGG AGGCCATTTC ACTCCCTGAA	2628
CCCAGCCTGA CAAATCACAG TGAGAAATGTT CACCTTATAG GCTTGCTGTG GGGCTCAGGT	2688
TGAAAGTGTG GGGAGTGACA CTGCCTAGGC ATCCAGCTCA GTGTCATCCA GGGCCTGTGT	2748 <sup>5/6</sup>
CCCTCCCGAA CCCAGGGTCA ACCTGCCTGC CACAGGCACT AGAAGGACGA ATCTGCCTAC	2808
TGCCCATGAA CGGGGCCCTC AAGCGTCCTG GGATCTCCTT CTCCCTCCTG TCCTGTCCTT	2868
GCCCCTCAGG ACTGCTGGAA AATAAATCCT TTAAATAAGT AAAAAAAAAA AAAA	2923

FIG. 1A-5

FIG. 1A

FIG. 1A-1
FIG. 1A-2
FIG. 1A-3
FIG. 1A-4
FIG. 1A-5

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Met	Ala	Arg	Leu	Gln	Thr	Ala	Leu	Leu	Val	Val	Leu	Val	Leu	Leu	Ala
-24				-20					-15					-10	
Val	Ala	Leu	Gln	Ala	Thr	Glu	Ala	Gly	Pro	Tyr	Gly	Ala	Asn	Met	Glu
			-5					1				5			
Asp	Ser	Val	Cys	Cys	Arg	Asp	Tyr	Val	Arg	Tyr	Arg	Leu	Pro	Leu	Arg
		10				15					20				
Val	Lys	His	Phe	Tyr	Tyr	Trp	Thr	Ser	Asp	Ser	Cys	Pro	Arg	Pro	Gly
25			30						35					40	
Val	Val	Leu	Leu	Thr	Phe	Arg	Asp	Lys	Glu	Ile	Cys	Ala	Asp	Pro	Arg
			45						50					55	
Val	Pro	Trp	Val	Lys	Met	Ile	Leu	Asn	Lys	Leu	Ser	Gln			
			60						65						

FIG. 1B



## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: Gallo, Robert C.  
DeVico, Anthony L.  
Garzino, Alfredo
- (ii) TITLE OF THE INVENTION: METHOD AND COMPOSITION TO ENHANCE  
THE EFFICACY OF A VACCINE USING MACROPHAGE DERIVED CHEMOKINE
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Pennie & Edmonds LLP  
(B) STREET: 1155 Avenue of the Americas  
(C) CITY: New York  
(D) STATE: New York  
(E) COUNTRY: USA  
(F) ZIP: 10036/2711
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: DOS  
(D) SOFTWARE: FastSEQ Version 2.0
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: To be assigned  
(B) FILING DATE: Herewith  
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Misrock, S. Leslie  
(B) REGISTRATION NUMBER: 18,872  
(C) REFERENCE/DOCKET NUMBER: 8769-029
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 212-790-9090  
(B) TELEFAX: 212-869-8864  
(C) TELEX: 66141 PENNIE
- (2) INFORMATION FOR SEQ ID NO:1:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2923 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(ix) FEATURE:  
(A) NAME/KEY: mat\_peptide  
(B) LOCATION: 92..298

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAGACATACA GGACAGAGC ATG GCT CGC CTA CAG ACT GCA CTC CTG GTT GTC	52
Met Ala Arg Leu Gln Thr Ala Leu Val	
-24 -20 -15	
CTC GTC CTC CTT GCT GTG GCG CTT CAA GCA ACT GAG GCA GGC CCC TAC	100
Leu Val Leu Ala Val Ala Leu Gln Ala Thr Glu Ala Gly Pro Tyr	
-10 -5 1	
GGC GCC AAC ATG GAA GAC AGC GTC TGC CGT GAT TAC GTC CGT TAC	148
Gly Ala Asn Met Glu Asp Ser Val Cys Arg Asp Tyr Val Arg Tyr	
5 10 15	
CGT CTG CCC CTG CGC GTG AAA CAC TTC TAC TGG ACC TCA GAC TCC	196
Arg Leu Pro Leu Arg Val Lys His Phe Tyr Trp Thr Ser Asp Ser	
20 25 30 35	
TGC CCG AGG CCT GGC GTG TTG CTA ACC TTC AGG GAT AAG GAG ATC	244
Cys Pro Arg Pro Gly Val Leu Thr Phe Arg Asp Lys Glu Ile	
40 45 50	
TGT GCC GAT CCC AGA GTG CCC TGG GTG AAG ATG ATT CTC AAT AAG CTG	292
Cys Ala Asp Pro Arg Val Pro Trp Val Lys Met Ile Leu Asn Lys Leu	
55 60 65	
AGC CAA TGAAGAGCCT ACTCTGATGA CCGTGCCTT GGCTCCTCCA GGAAGGCTCA	348
Ser Gln	

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GGAGCCCTAC CTCCCTGCCA TTATAGCTGC TCCCCGCCAG AAGCCTGTGC CAACTCTCTG 408
CATTCCCTGA TCTCCATCCC TGTGGCTGTC ACCCTTGGTC ACCTCCGTGC TGTCAGTGCC 468
ATCTCCCCCC TGACCCCTCT AACCCATCCT CTGCCTCCCT CCCTGCAGTC AGAGGGTCCT 528
GTTCCCATCA GCGATTCCCC TGCTTAAACC CTTCATGAC TCCCCACTGC CCTAAGCTGA 588
GGTCAGTCTC CCAAGCCTGG CATGTGGCCC TCTGGATCTG GGTTCATCT CTGTCTCCAG 648
CCTGCCCACT TCCCTTCATG AATGTTGGGT TCTAGCTCCC TGTTCCTCAA ACCCATACTA 708
CACATCCCAT TTCTGGGTCT TTGCCTGGGA TGTGCTGAC ACTCAGAAAG TCCCACCACC 768
TGCACATGTG TAGCCCCACC AGCCCTCCAA GGCATTGCTC GCCCCAAGCAG CTGGTAATTC 828
CATTTCATGT ATTAGATGTC CCCCTGGCCCT CTGTCCCTC TTAATAACCC TAGTCACAGT 888
CTCCGCAGAT TCTTGGGATT TGGGGGTTTT CTCCCCACC TCTCCACTAG TTGGACCAAG 948
GTTTCTAGCT AAGTTACTCT AGTCTCCAAG CCTCTAGCAT AGAGCACTGC AGACAGGCCC 1008
TGGCTCAGAA TCAGAGCCCA GAAAGTGGCT GCAGACAAAA TCAATAAAAC TAATGTCCCT 1068
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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C-CHEMOKINES  
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(SCM-1)

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CX3C-chemokines  
Fractalkine/neurotactin

U91835 U84487

LOCUS HSU83171 2923 bp mRNA PRI 31-MAY-1997  
 DEFINITION Human macrophage-derived chemokine precursor (MDC) mRNA,  
 complete cds.  
 ACCESSION U83171  
 NID g1931580  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;  
 Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 2923)  
 AUTHORS Godiska, R., Chantry, D., Raport, C.J., Sozzani, S., Allavena, P.,  
 Leviten, D., Mantovani, A. and Gray, P.W.  
 TITLE Human macrophage-derived chemokine (MDC), a novel  
 chemoattractant for monocytes, monocyte-derived dendritic cells, and natural  
 killer cells  
 JOURNAL J. Exp. Med. 185 (9), 1595-1604 (1997)  
 MEDLINE 97296313  
 REFERENCE 2 (bases 1 to 2923)  
 AUTHORS Godiska, R. and Gray, P.W.  
 TITLE Direct Submission  
 JOURNAL Submitted (23-DEC-1996) ICOS Corporation, 22021 20th Avenue SE,  
 Bothell, WA 98021, USA  
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DEFINITION Human CC chemokine STCP-1 mRNA, complete cds.
ACCESSION  U83239
NID        g2062424
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 932)
AUTHORS    Chang,M.S., McNinch,J., Elias III,C., Manthey,C.L.,
Grosshans,D.,
            Meng,T., Boone,T. and Andrew,D.P.
TITLE      Molecular cloning and functional characterization of a novel CC
            chemokine STCP-1 which specifically acts on activated T
            lymphocytes
JOURNAL     Unpublished
REFERENCE  2 (bases 1 to 932)
AUTHORS    Chang,M.S., McNinch,J., Elias III,C., Manthey,C.L.,
Grosshans,D.,
            Meng,T., Boone,T. and Andrew,D.P.
TITLE      Direct Submission
JOURNAL     Submitted (26-DEC-1996) Research Computing, Amgen Institute,
620
            University Ave, Suite 706, Toronto, ON M5G 2C1, Canada
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ACCESSION  X14768
NID        g34513
KEYWORDS   monocyte chemoattractant protein 1.
SOURCE     human.
ORGANISM   Homo sapiens
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REFERENCE  1 (bases 1 to 725)
AUTHORS    Yoshimura, T., Yuhki, N., Moore, S.K., Appella, E., Lerman, M.I. and
            Leonard, E.J.
TITLE      Human monocyte chemoattractant protein-1 (MCP-1). Full-length
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            cloning, expression in mitogen-stimulated blood mononuclear
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JOURNAL    FEBS Lett. 244 (2), 487-493 (1989)
MEDLINE    89153605
COMMENT     ZAP11.
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DEFINITION H.sapiens MCP-2 gene.
ACCESSION  X99886
NID        g1905800
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SOURCE     human.
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 2991)
AUTHORS    Van Coillie, E., Fiten, P., Nomiya, H., Sakaki, Y., Miura, R.,
            Yoshie, O., Van Damme, J. and Opdenakker, G.
TITLE      The human MCP-2 gene (SCYA8): cloning, sequence analysis,
tissue     expression, and assignment to the CC chemokine gene contig on
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JOURNAL     Genomics 40 (2), 323-331 (1997).
MEDLINE     97237052
REFERENCE  2 (bases 1 to 2991)
AUTHORS    Opdenakker, G.M.M.
TITLE      Direct Submission
JOURNAL     Submitted (07-AUG-1996) G.M.M. Opdenakker, Rega Institute for
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SOURCE       human.
ORGANISM     Homo sapiens
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              Primates; Catarrhini; Hominidae; Homo.
REFERENCE    1 (bases 1 to 1368)
AUTHORS      Van Coillie,E.
TITLE        Functional comparison of two human monocyte chemotactic
protein-2
              isoforms, role of the amino-terminal pyroglutamic acid and
              processing by CD26/dipeptidyl peptidase IV
              Biochemistry 37, 12672-12680 (1998)
REFERENCE    2 (bases 1 to 1368)
AUTHORS      Van Coillie,E.
TITLE        Direct Submission
JOURNAL      Submitted (23-FEB-1998) E. Van Coillie, Rega Institute for
Medical
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COMMENT      Related sequences: X99886, Y10802.
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  301 gcttactcag catattcaag gaaggtctta ctccattctt ccttgattgt gaccatgccc
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DEFINITION   H.sapiens MCP-3 mRNA for monocyte chemotactic protein-3.
ACCESSION    X72308 S57464
NID          g313707
KEYWORDS     monocyte chemotactic protein 3.
SOURCE       human.
ORGANISM     Homo sapiens
              Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
              Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE    1 (bases 1 to 1085)
AUTHORS      Opdenakker,G., Froyen,G., Fiten,P., Proost,P. and Van Damme,J.
TITLE        Human monocyte chemotactic protein-3 (MCP-3): molecular cloning
of           the cDNA and comparison with other chemokines
              Biochem. Biophys. Res. Commun. 191 (2), 535-542 (1993)
JOURNAL      93213290
MEDLINE
REFERENCE    2 (bases 1 to 1085)
AUTHORS      Opdenakker,G.M.
TITLE        Direct Submission
JOURNAL      Submitted (27-MAY-1993) G.M. Opdenakker, Rega Institute,
University   of Leuven, Minderbroedersstraat 10, B-3000 Leuven, BELGIUM
REFERENCE    3 (bases 1 to 1085)
AUTHORS      Opdenakker,G., Fiten,P., Nys,G., Froyen,G., Van Roy,N.,
              Speleman,F., Laureys,G. and Van Damme,J.
TITLE        The human MCP-3 gene (SCYA7): cloning, sequence analysis, and
              assignment to the C-C chemokine gene cluster on chromosome
              17q11.2-q12
JOURNAL      Genomics 21 (2), 403-408 (1994)
MEDLINE      94375065
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LOCUS      HSMCP3A      1085 bp      DNA      PRI      25-JUL-1994
DEFINITION H.sapiens MCP-3 mRNA for monocyte chemotactic protein-3.
ACCESSION  X72308 S57464
NID        g313707
KEYWORDS   monocyte chemotactic protein 3.
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 1085)
AUTHORS    Opdenakker,G., Froyen,G., Fiten,P., Proost,P. and Van Damme,J.
TITLE      Human monocyte chemotactic protein-3 (MCP-3): molecular cloning
of         the cDNA and comparison with other chemokines
JOURNAL    Biochem. Biophys. Res. Commun. 191 (2), 535-542 (1993)
MEDLINE    93213290
REFERENCE  2 (bases 1 to 1085)
AUTHORS    Opdenakker,G.M.
TITLE      Direct Submission
JOURNAL    Submitted (27-MAY-1993) G.M. Opdenakker, Rega Institute,
University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, BELGIUM
REFERENCE  3 (bases 1 to 1085)
AUTHORS    Opdenakker,G., Fiten,P., Nys,G., Froyen,G., Van Roy,N.,
            Speleman,F., Laureys,G. and Van Damme,J.
TITLE      The human MCP-3 gene (SCYA7): cloning, sequence analysis, and
            assignment to the C-C chemokine gene cluster on chromosome
            17q11.2-q12
JOURNAL    Genomics 21 (2), 403-408 (1994)
MEDLINE    94375065
FEATURES   Location/Qualifiers
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961 aattgttaag atatgatgtc cctatggaag catattgtta ttatataatt acatatttgc
1021 atatgtatga ctcccaaatt ttcacataaa atagattttt gtataacaaa aaaaaaaaaa
1081 aaaaa

//LOCUS      HSU46767      825 bp      mRNA      PRI      16-DEC-1996
DEFINITION   Human monocyte chemoattractant protein-4 precursor (MCP-4)
mRNA,
complete cds.
ACCESSION    U46767
NID          gl732122
KEYWORDS
SOURCE       human.
ORGANISM     Homo sapiens
              Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
              Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE    1 (bases 1 to 825)
AUTHORS      Garcia-Zepeda, E.A., Combadiere, C.C., Rothenberg, M.E.,
Sarafi, M.N.,
              Lavigne, F., Hamid, Q., Murphy, P. and Luster, A.D.
TITLE        Human monocyte chemoattractant Protein (MCP)-4: A novel CC
              chemokine with activities on monocytes, eosinophils, and
basophils
              induced in allergic and non-allergic inflammation that signals
              through the CC chemokine receptors CCR-2 and 3
JOURNAL      J. Immunol. 158 (1996) In press
REFERENCE    2 (bases 1 to 825)
AUTHORS      Garcia-Zepeda, E.A. and Luster, A.D.
TITLE        Direct Submission
JOURNAL      Submitted (22-JAN-1996) Eduardo A. Garcia-Zepeda, Infectious
              Disease Unit, Massachusetts General Hospital, 149 13th St.,
              Charlestown, MA 02129, USA
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//LOCUS HSAMAC1 803 bp RNA PRI 10-AUG-1997  
DEFINITION Homo sapiens mRNA for alternative activated macrophage specific CC

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ACCESSION Y13710  
NID g2326515  
KEYWORDS AMAC-1 gene; CC-chemokine 1.  
SOURCE human. ORGANISM Homo sapiens  
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;

Hominidae;

Homo.  
REFERENCE 1 (bases 1 to 803)  
AUTHORS Politz,O.  
TITLE Direct Submission  
JOURNAL Submitted (10-JUN-1997) Politz O., Dermatology, Free University  
Benjamin Franklin Medical Center, Hindenburgdamm 30; 12200  
Berlin

GERMANY  
REFERENCE 2 (bases 1 to 803)  
AUTHORS Kodelja,V., Mueller,C., Politz,O., Hakiy,N., Orfanos,C.E. and  
Goerdts,S.  
TITLE Cloning of alternative activated macrophage associated CC  
chemokine

1 (AMAC-1)  
JOURNAL Unpublished

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781 aaaaaaaaaa aaaaaaaaaa aaa

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LOCUS       HUMLD78A       3176 bp      DNA              PRI      17-JAN-1992
DEFINITION   Human LD78 alpha gene.
ACCESSION    D90144
NID          g219905
KEYWORDS     LD78; LD78 alpha; cytokine; inducible gene family; secreted
             peptide.
SOURCE       Human blood lymphocyte DNA, clone Lm LD-3.
ORGANISM     Homo sapiens
             Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
             Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae;   Homo.
REFERENCE    1 (bases 1 to 3176)
AUTHORS      Nakao,M.; Nomiya,H. and Shimada,K.
TITLE        Structures of human genes coding for cytokine LD78 and their
             expression
JOURNAL      Mol. Cell. Biol. 10 (7), 3646-3658 (1990)
MEDLINE      90287155
COMMENT      These data kindly submitted in computer readable form by:
Hisayuki

```

```

Nomiya
Department of Biochemistry
Kumamoto University Medical School
2-2-1 Honjo, Kumamoto 860
Japan
Phone: 096-344-2111
Fax: 096-372-6140.

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     sig_peptide        1155..1220
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                       /codon_start=1
                       /product="LD78 alpha precursor"
                       /db_xref="PID:d1014875"
                       /db_xref="PID:g219906"
/translation="MQVSTAALAVLLCTMALCNQFSASLAADTPTACCFSTSRQIPQ
             NFIADYFETSSQCSKPGVIFLTKRSRQVCADPSEEWQKYVSDLELSA"
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                       /note="LD78 alpha mature peptide"
     intron             1228..1915
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121  atatatgtgt  atatgtacaa  atacatgtat  atatagtaca  aatatatata  tatatttgta
181  caattcttca  gactttgtag  aatttgtata  atgtcgtatc  ttgttttttt  taaccactga
241  tgttataagc  atatttatgc  cacttcattc  attttagaga  cttaataata  aatgatctag
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421  agaactcttt  cctagaaatg  ctttttctag  gttaatggct  acagatattt  ctaggcacct
481  gacataattg  caccacacct  taaagtattt  ttatgatcca  caactagcgt  ttaacacagc
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601  tattcctcca  gctttcattc  agttctttgc  ctctgggagg  aggaagggtt  gtgcagccct
661  ccacagcatc  agcccatcaa  ccctatccct  gtgggttatg  cagctgagga  agcagaattg
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781  ggactgacta  agaatagcct  tgggttgaca  tatacccttc  ttcacactca  caggagaaac
841  catttcccta  tgaaactata  acaagtcata  agttgagagc  tgagagttag  agaataagctc
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1021  ccccatccgc  cagggtgccc  tataaagagg  agagctgggt  tcagacttca  gaaggacacg
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1321  agagaacttc  ttaaagggct  gccaaacatc  ttggtctttc  tctttaagac  ttttattttt
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1561  taggtctccc  aggagctctg  tccttggat  gtcttatgag  agatgtccaa  ggcttctctt
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1741  ggagaaagaa  ggatacagac  agaaacaaag  agacatttct  cagcaaaaat  gcccaaatgc
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1981  atagctgact  actttgagac  gagcagccag  tgctccaagc  cgggtgtcat  gtaagtcca
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2101  aaggctatcc  tggaaaggcc  cagccttcag  gagcctatcg  gggatagagg  acgcagggct
2161  ccgaggtgtg  acctgacttg  gagctggagt  gaggcattgt  ttacagagtc  aggaagggtt
2221  gccccagccc  agaggaaagg  gacaggaaga  aggaggcagc  gggacactct  gagggccacc
2281  cctactgagt  cactgagaga  agctctctag  acagagatag  gcaggggggc  cctgaaagag
2341  gagcaagccc  tgagctgccc  aggacagaga  gcagaatggt  gggggccatg  tggggccagg
2401  attccccctg  tggattcccc  agtgcttaac  tcttctctcc  ttctccacag  ctctcctaac
2461  aagcgaagcc  ggcaggtctg  tgctgacccc  agtgaggagt  ggggtccaga  atatgtcagc
2521  gacctggagc  tgagtgcctg  aggggtccag  aagcttcgag  gccagcgac  ctcgggtggc
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2881  aaatgtgtat  cggatgcttt  tgttcagggc  tgtgatcggc  ctggggaaat  aataaagatg
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3001  ctggttaaga  ggaatcatag  gcaaagatta  ggaagagggt  aaatggaggg  aaattggggg
3061  agatggggag  ggctaccaca  gagttatcca  ctttacaacg  gagacacagt  tctggaacat
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LOCUS      AF043339      225 bp      mRNA      PRI      23-FEB-1998
DEFINITION Homo sapiens macrophage inflammatory protein 1 alpha (MIP1a)
mRNA,
partial cds.
ACCESSION  AF043339
NID        g2905627
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 225)
AUTHORS    Jang, J.S. and Kim, B.E.
TITLE      Direct Submission
JOURNAL    Submitted (15-JAN-1998) Protein Engineering, General Institute

```

of  
Technology, Hyundai Pharm. Ind. Co., Ltd., 213 Sosa Bon 1-dong,  
Sosa-gu, Bucheon 422-231, Korea

COMMENT forward primer (5'-tgcgcatcacttgctgctgaca-3')  
reverse primer (5'-cttctggaccctcaggcact-3').

FEATURES Location/Qualifiers  
source 1..225  
/organism="Homo sapiens"  
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gene <1..225  
/gene="MIP1a"  
primer\_bind <1..19  
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/PCR\_conditions="94C-1min, 50C-1min, 72C-3min, 30  
cycles;  
CDS DeltaCycler II from Ericomp"  
<1..213  
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/function="proinflammatory cytokine involved in  
inflammation"  
/note="8-10 kDa"  
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/product="macrophage inflammatory protein 1 alpha"  
/db\_xref="PID:g2905628"

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BASE COUNT 50 a 68 c 62 g 45 t  
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61 ccacagaatt tcatagctga ctactttgag acgagcagcc agtgctccaa gcccggtgct  
121 atctttcctaa ccaagcgaag ccggcagggtc tgtgctgacc ccagtgagga gtgggtccag  
181 aaatatgtca gcgacctgga gctgagtgcc tgaggggtcc agaag.

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LOCUS HUMLD78B 3112 bp DNA PRI 17-JAN-1992  
DEFINITION Human LD78 beta gene.  
ACCESSION D90145  
NID g219907  
KEYWORDS LD78; LD78 beta; cytokine; inducible gene family; secreted  
peptide.  
SOURCE Human placenta DNA, clone Lm LD-1.  
ORGANISM Homo sapiens  
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;  
Hominidae;  
Homo.  
REFERENCE 1 (bases 1 to 3112)  
AUTHORS Nakao,M., Nomiyama,H. and Shimada,K.  
TITLE Structures of human genes coding for cytokine LD78 and their  
expression  
JOURNAL Mol. Cell. Biol. 10 (7), 3646-3658 (1990)  
MEDLINE 90287155  
COMMENT These data kindly submitted in computer readable form by:  
Hisayuki  
Nomiyama  
Department of Biochemistry  
Kumamoto University Medical School  
2-2-1 Honjo, Kumamoto 860  
Japan  
Phone: 096-344-2111  
Fax: 096-372-6140.

FEATURES Location/Qualifiers  
source 1..3112  
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/db\_xref="taxon:9606"  
repeat\_unit 498..797  
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CDS              join(1192..1267,1953..2067,2488..2578)
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                  /product="LD78 beta precursor"
                  /db_xref="PID:d1014876"
                  /db_xref="PID:g219908"

/translation="MQVSTAALAVLLCTMALCNQVLSAPLAADPTACCFSYTSRQIP
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exon             1953..2067
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exon             2488..2955
                  /number=3

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121 atggctccat atttgggttg tttccacaga actctttccc agaaatgctt tttctaggtt
181 aatgggtaca catatttcta ggcacctgac atactgacac ccacctctaa agtattttta
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421 gttatagcag ctgaggaagc agaattacag ctctgtggga aggaatgggg ctggagaggtt
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901 tcatgagttg agagctgaga gttagagagt agctcagaga tgctattctt ggatattctg
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2521 tgacccagct gaggagtggg tccagaaata cgtcagtgac ctggagctga gtgcctgagg
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2641 gagccttggg aacatgcgtg tgacctctac agctacctct tctatggact ggttattgcc
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LOCUS      HUMACT2A      696 bp      mRNA      PRI      30-OCT-1994
DEFINITION Human activation (Act-2) mRNA, complete cds.
ACCESSION  J04130
NID        g178017
KEYWORDS   act2 gene; immune activation gene.
SOURCE     Human (Hut-102B2 library) activated T cells, cDNA to mRNA.
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 696)
AUTHORS    Lipas,M.A., Napolitano,M., Jeang,K.T., Chang,N.T. and
Leonard,W.J.
  TITLE     Identification, cloning, and characterization of an immune
            activation gene
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 85 (24), 9704-9708 (1988)
  MEDLINE   89071764
  COMMENT   Draft entry and computer-readable sequence [1] kindly submitted
            by
            W.Leonard, 09-JAN-1989.
FEATURES   Location/Qualifiers
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ORIGIN           Unreported.

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121 gtgactgtcc tgtctctcct catgctagta gctgccttct gctctccagc gctctcagca
181 ccaatgggct cagaccctcc caccgcctgc tgettttctt acaccgcgag gaagcttccct
241 cgcaactttg tggtagatta ctatgagacc agcagcctct gctccccagc agctgtggtta
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361 tacgtgtatg acctggaact gaactgagct gctcagagac aggaagtctt caggaaggt
421 cacctgagcc cggatgcttc tccatgagac acatctcttc catactcagg actcctctcc
481 gcagttcctg tcccttctct taatttaatc ttttttatgt gccgtgttat tgtattaggt
541 gtcatttcca ttatttatat tagtttagcc aaaggataag tgtcctatgg ggtatggcca
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661 ccataataaa actttaaaat aaaatgcaga cagtta

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LOCUS      AF031587      481 bp      mRNA      PRI      02-JAN-1998
DEFINITION Homo sapiens MIP-1 delta mRNA, complete cds.

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ACCESSION AF031587  
 NID g2739163  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;  
 Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 481)  
 AUTHORS Wang, W.  
 TITLE Molecular cloning and characterization of a new CC chemokine  
 MIP-1  
 JOURNAL delta  
 Unpublished  
 REFERENCE 2 (bases 1 to 481)  
 AUTHORS Wang, W.  
 TITLE Direct Submission  
 JOURNAL Submitted (27-OCT-1997) Immunobiology, DNAX Research Institute,  
 901  
 California Ave, Palo Alto, CA 94304, USA  
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 DCMKKLKPYSI"  
 BASE COUNT 140 a 112 c 100 g 129 t  
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 61 gccagttca taaatgatgc agagacagag ttaatgatgt caaagcttcc actggaaaat  
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 181 agcatcccggt gttcactcat gaaaagttat tttgaaacga gcagcgagtg ctccaagcca  
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 301 gttcaggatt gcatgaaaaa gctgaagccc tactcaatat aataataaag agacaaaaga  
 361 gggcagccac ccacctccaa cactcctgt gagtttcttg gtctgaaata cttaaaaaat  
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 481 t  
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 LOCUS AF043340 234 bp mRNA PRI 23-FEB-1998  
 DEFINITION Homo sapiens macrophage inflammatory protein 2 alpha (MIP2a)  
 mRNA,  
 partial cds.  
 ACCESSION AF043340  
 NID g2905629  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;  
 Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 234)  
 AUTHORS Jang, J.S. and Kim, B.E.  
 TITLE Direct Submission  
 JOURNAL Submitted (15-JAN-1998) Protein Engineering, General Institute  
 of  
 Technology, Hyundai Pharm. Ind. Co., Ltd., 213 Sosa Bon 1-dong,  
 Sosa-gu, Bucheon 422-231, Korea  
 COMMENT forward primer (5'-tgcgacccctggccactgaactg-3')  
 reverse primer (5'-ccttcctctgtggtcagttgga-3').  
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 /db\_xref="taxon:9606"  
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CDS           <1..222
              /gene="MIP2a"
              /function="CXC chemokine"
              /function="proinflammatory cytokine involved in
inflammation"
              /note="8-10 kDa"
              /codon_start=1
              /product="macrophage inflammatory protein 2 alpha"
              /db_xref="PID:g2905630"

/translation="APLATELRCQCLQTLQGIHLKNIQSVKVKSPGPHCAQTEVIATL
              KNGQKACLNPA SP MVKKIIEKMLKNGKSN"
primer_bind   complement(214..234)
              /gene="MIP2a"
BASE COUNT    74 a      70 c      54 g      36 t
ORIGIN
1 gcacccctgg ccactgaact gcgctgccag tgcttcgaga ccctgcaggg aattcacctc
61 aagaacatcc aaagtgtgaa ggtgaagtcc cccggacccc actgcgcccc aaccgaagtc
121 atagccacac tcaagaatgg gcagaaagct tgtctcaacc ccgcatcgcc catgggtaaag
181 aaaatcatcg aaaagatgct gaaaaatggc aaatccaact gaccagaagg aagg
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LOCUS          HSU77035      764 bp      mRNA          PRI      23-JAN-1997
DEFINITION     Human macrophage inflammatory protein 3 alpha (MIP-3a) mRNA,
complete cds.
ACCESSION      U77035
NID            g1790924
KEYWORDS
SOURCE         human.
ORGANISM       Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE      1 (bases 1 to 764)
AUTHORS        Rossi,D.L., Vicari,A.P., Franz-Bacon,K., McClanahan,T.K. and
Zlotnik,A.
TITLE          Identification through bioinformatics of two new macrophage
proinflammatory human chemokines: MIP-3alpha and MIP-3beta
JOURNAL        J. Immunol. 158 (3), 1033-1036 (1997)
MEDLINE        97166046
REFERENCE      2 (bases 1 to 764)
AUTHORS        Rossi,D.L. and Zlotnik,A.
TITLE          Direct Submission
JOURNAL        Submitted (31-OCT-1996) Immunology, DNAX Research Institute,
901
California Ave., Palo Alto, CA 94304, USA
FEATURES
source         Location/Qualifiers
              1..764..
              /organism="Homo sapiens"
              /db_xref="taxon:9606"
              /cell_type="elutriated monocytes activated with
LPS/IFN-GAMMA"
gene          1..291
              /gene="MIP-3a"
CDS           1..291
              /gene="MIP-3a"
              /note="chemokine"
              /codon_start=1
              /product="macrophage inflammatory protein 3 alpha"
              /db_xref="PID:g1790925"

/translation="MCCTKSLLLAALMSVLLHLGGESEASNFDCCLGYTDRILHPK
              FIVGFTRQLANEGCDINAIIFHTKKKLSVCANPKQTWVKYIVRLLSKVKVNM"
BASE COUNT    235 a      121 c      146 g      260 t      2 others
ORIGIN
1 atgtgctgta ccaagagttt gctcctggct gctttgatgt cagtgtgct actccacctc

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61  tgcggcgaat  cagaagcagc  aagcaacttt  gactgctgtc  ttggatacac  agaccgtatt
121 cttcatccta  aattttattgt  gggcttcaca  cggcagctgg  ccaatgaagg  ctgtgacatc
181 aatgctatca  tctttcacac  aaagaaaaag  ttgtctgtgt  gcgcaaatcc  aaaacagact
241 tgggtgaaat  atattgtgcg  tctcctcagt  aaaaaagtca  agaacatgta  aaaactgtgg
301 cttttctgga  atggaattgg  acatagccca  agaacagaaa  gaaccttgct  ggggttggag
361 gtttcacttg  cacatcatgg  agggtttagt  gcttatctaa  tttgtgcctc  actggacttg
421 tccaattaat  gaagttgatt  catattgcat  catagtttgc  tttgtttaag  catcacatta
481 aagttaaact  gtattttatg  ttatttatag  ctgtaggttt  tctgtgttta  gctatttaac
541 actaattttc  cataagctat  tttggtttag  tgcaaagtat  aaaattatat  ttggggggga
601 ataagattat  atggactttt  ttgcaagcaa  caagctattt  tttaaaamma  actatttaac
661 attcttttgt  ttatatgttt  ttgtctccta  aattgttgta  attgcattat  aaaataagaa
721 aaatattaat  aagacaaata  ttgaaaataa  agaaacaaaa  agtt

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LOCUS      HSU77180      545 bp      mRNA      PRI      23-JAN-1997
DEFINITION Human macrophage inflammatory protein 3 beta (MIP-3beta) mRNA,
complete cds.
ACCESSION  U77180
NID        gl791002
KEYWORDS
SOURCE     human:
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 545)
AUTHORS    Rossi,D.L., Vicari,A.P., Franz-Bacon,K., McClanahan,T.K. and
            Zlotnik,A.
TITLE       Identification through bioinformatics of two new macrophage
            proinflammatory human chemokines: MIP-3alpha and MIP-3beta
JOURNAL     J. Immunol. 158 (3), 1033-1036 (1997)
MEDLINE     97166046
REFERENCE  2 (bases 1 to 545)
AUTHORS    Vicari,A. and Zlotnik,A.
TITLE       Direct Submission
JOURNAL     Submitted (01-NOV-1996) Immunology, DNAX Research Institute,
901         California Ave, Palo Alto, CA 94304, USA
FEATURES
            Location/Qualifiers
            source      1..545
                        /organism="Homo sapiens"
                        /db_xref="taxon:9606"
                        /cell_type="macrophages activated with LPS or IFNg"
                        /chromosome="9"
            gene        1..297
                        /gene="MIP-3beta"
            CDS          1..297
                        /gene="MIP-3beta"
                        /function="chemokine"
                        /codon_start=1
                        /product="macrophage inflammatory protein 3 beta"
                        /db_xref="PID:gl791003"

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/translation="MALLLALSLVLWTSPAPTLSGTNDACCLSVTQKPIPGYIVR

NFHYLLIKDGRVPAVVFTTLRGRQLCAPDPWVERIIQRLQRTSAKMKRRSS"

BASE COUNT 125 a 160 c 153 g 107 t

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ORIGIN
1  atggccctgc  tactggccct  cagcctgctg  gttctctgga  cttccccagc  cccaactctg
61  agtggcacca  atgatgctga  agactgctgc  ctgtctgtga  cccagaaacc  catccctggg
121  tacatcgtga  ggaacttcca  ctaccttctc  atcaaggatg  gctgcagggt  gcctgctgta
181  gtgttcacca  cactgagggg  ccgccagctc  tgtgcacccc  cagaccagcc  ctgggtagaa
241  cgcatcatcc  agagactgca  gaggacctca  gccaaagtga  agcgccgcag  cagttaacct
301  atgaccgtgc  agagggagcc  cggagtccga  gtcaagcatt  gtgaattatt  acctaacctg
361  gggaaaccgag  gaccagaagg  aaggaccagg  cttccagctc  ctctgcacca  gacctgacca
421  gccaggacag  ggcctggggg  gtgtgtgagt  gtgagtgtga  gcgagagggt  gagtgtggtc
481  tagagtaaa  ctgctccacc  cccagattgc  aatgctacca  ataaagccgc  ctgggtgtta
541  caact

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LOCUS      HUMTCSM      1160 bp      mRNA      PRI      15-JUN-1989
DEFINITION Human T cell-specific protein (RANTES) mRNA, complete cds.
ACCESSION  M21121
NID        g339420

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KEYWORDS Alu repeat; T-cell-specific protein.  
 SOURCE Human peripheral blood (T lymphocyte) cell line AH2, cDNA to mRNA,  
 ORGANISM clone 228.  
 Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 1160)  
 AUTHORS Schall,T.J., Jongstra,J., Dyer,B.J., Jorgensen,J.,  
 Clayberger,C., Davis,M.M. and Krensky,A.M.  
 TITLE A human T cell-specific molecule is a member of a new gene family  
 JOURNAL J. Immunol. 141, 1018-1025 (1988)  
 MEDLINE 88285659  
 COMMENT Draft entry and computer-readable sequence for [1] kindly provided by A.M.Krensky, 24-OCT-1988.

FEATURES  
 source Location/Qualifiers  
 1..1160  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 CDS 27..302  
 /note="T cell-specific protein precursor"  
 /codon\_start=1  
 /db\_xref="PID:g339421"

/translation="MKVSAARLAVILIATALCAPASAPYSSDTPCCFAYIARPLPR  
 AHIEKFYFTSGKCSNPVVFVTRKNRQVCANPEKKWVREYINSLEMS"  
 sig\_peptide 27..95  
 /note="T cell-specific protein signal peptide"  
 mat\_peptide 96..299  
 /note="T cell-specific protein"  
 repeat\_region 450..950  
 /note="Alu-related repeats"

BASE COUNT 298 a 332 c 295 g 235 t

ORIGIN 276 bp upstream of RsaI site.

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1 cctccgacag cctctccaca ggtaccatga aggtctccgc ggcacgcctc gctgtcatcc
61 tcattgctac tgccctctgc gtccttgcac ctgcctcccc atattctctg gacaccacac
121 cctgtgtgctt tgccctacatt gcccgccac tgccccgtgc ccacatcaag gagtatttct
181 acaccagtgg caagtgtctcc aaccagcag tcgtctttgt caccgaaag aaccgccaag
241 tgtgtgccaa cccagagaag aaatgggttc gggagtacat caactcttgg gagatgagct
301 aggatggaga gtccttgaac ctgaacttac acaaatttgc ctgtttctgc ttgctcttgt
361 cctagcttgg gaggcttccc ctactatcc taccacccc gctccttgaa gggcccagat
421 tctgaccacg acgagcagca gttacaaaaa ccttccccag gctggacgtg gtggctcagc
481 cttgtaatcc cagcactttg ggaggccaag gtgggtggat cacttgaggt caggagttcg
541 agacagcctg gccaacatga tgaaacccca tgtgtactaa aaatacaaaa aattagccgg
601 gcgtggtagc gggcgctgt agtcccagct actcgggagg ctgaggcagg agaattggct
661 gaacccggga gcggagcttg cagtgaagcg agatcgcgcc actgcactcc agcctgggag
721 acagagcgag actccgtctc aaaaaaaaaa aaaaaaaaaa aaaaaatata aaaattagcc
781 gcgtgggtgg ccacgcctgt aatcccagct actcgggagg ctaaggcagg aaaattgttt
841 gaacccagga ggtggaggct gcagtgaact gagattgtgc cacttcactc cagcctgggt
901 gacaaagtga gactccgtca caacaacaac aacaaaaagc ttccccaact aaagcctaga
961 agagcttctg aggcgtctgt ttgtcaaaag gaagtctcta gggtctgagc tctggcttgg
1021 ccttggcttt gcaagggctc tgtgacaagg aaggaagtca gcatgcctct agaggcaagg
1081 aaggaggagg cactgcactc ttaagcttcc gccgtctcaa cccctcacag gagcttactg
1141 gcaaacatga aaatcgggg

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LOCUS HUMTLI309 520 bp mRNA PRI 14-JAN-1995  
 DEFINITION Human secreted protein (I-309) mRNA, complete cds.  
 ACCESSION M57502  
 NID g339728  
 KEYWORDS secreted protein.  
 SOURCE Human T-cell, cDNA to mRNA.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 520)  
 AUTHORS Miller,M.D., Hata,S., De Waal Malefyt,R. and Krangel,M.S.  
 TITLE A novel polypeptide secreted by activated human T lymphocytes  
 JOURNAL J. Immunol. 143 (9), 2907-2916 (1989)

MEDLINE 90038522

FEATURES  
 source 1..520  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 /cell\_type="T-cell"  
 /germline  
 /map="17"  
 mRNA <1..520  
 /gene="SCYA1"  
 /note="G00-118-872"  
 gene 1..520  
 /gene="SCYA1"  
 CDS 51..341  
 /gene="SCYA1"  
 /codon\_start=1  
 /db\_xref="GDB:G00-118-872"  
 /product="secreted protein I-309"  
 /db\_xref="PID:g339729"

/translation="MQIITLALVCLLLAGMWPEDVDSKSMQVPFSCCFSAEQEIPL  
 RAILCYRNTSSICSNEGLIFKLKRGKEACALDTVGWVQRHRKMLRHCPSKRK"

BASE COUNT 140 a 137 c 122 g 121 t  
 ORIGIN

1 accaggtca tcaaagctgc tccaggaagg cccaagccag accagaagac atgcagatca  
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 121 agagcatgca ggtacccttc tccagatggt gcttctcatt tgcggagcaa gagattcccc  
 181 tgagggaat cctgtgttac agaaatacca gctccatctg ctccaatgag ggcttaatat  
 241 tcaagctgaa gagaggcaaa gaggcctgcg ccttggacac agttggatgg gtccagaggg  
 301 acagaaaaat gctgaggcac tgcccgtaaa aaagaaaatg agcagatttc ttccattgt  
 361 gggctctgga aaccacatgg cttcacctgt ccccgaaact accagcccta caccattcct  
 421 tctgccctgc ttttgctagg tcacagagga tctgcttggt cttgataagc tatgttggtg  
 481 cactttaaac atttaaatta tacaatcatc aacccccaac

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LOCUS AB000887 687 bp mRNA PRI 05-JUN-1997  
 DEFINITION Human mRNA for EB11-ligand chemokine, complete cds.  
 ACCESSION AB000887  
 NID g2189952  
 KEYWORDS EB11-ligand chemokine; ELC.  
 SOURCE Homo sapiens fetal tissue\_lib:lung cDNA to mRNA.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;

Hominidae;

Homo.

REFERENCE 1 (bases 1 to 687)

AUTHORS Yoshida,R., Imai,T., Hieshima,K., Kusuda,J., Baba,M.,  
 Kitaura,M.,

Nishimura,M., Kakizaki,M., Nomiyama,H. and Yoshie,O.

TITLE Direct Submission

JOURNAL Submitted (05-FEB-1997) to the DDBJ/EMBL/GenBank databases.

Hisayuki Nomiyama, Kumamoto University Medical School,

Department

of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan  
 (E-mail:nomiyama@gpo.kumamoto-u.ac.jp, Tel:+81-96-373-5063)

REFERENCE 2 (sites)

AUTHORS Yoshida,R., Imai,T., Hieshima,K., Kusuda,J., Baba,M.,  
 Kitaura,M.,

Nishimura,M., Kakizaki,M., Nomiyama,H. and Yoshie,O.

TITLE Molecular cloning of a novel human CC chemokine EB11-ligand  
 chemokine that is a specific functional ligand for EB11, CCR7

J. Biol. Chem. 272 (21), 13803-13809 (1997)

MEDLINE 97298088

FEATURES Location/Qualifiers

source 1..687  
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 /tissue\_lib="lung"  
 gene 139..435  
 /gene="ELC"

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CDS              139..435
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                  /note="CC chemokine"
                  /codon_start=1
                  /product="EBI1-ligand chemokine"
                  /db_xref="PID:d1021215"
                  /db_xref="PID:g2189953"

/translation="MALLLLALLVLTSPAPTLSGTNDACCLSVTQKPIPGYIVR
NFHYLLIKDGRVPAVVFTTLRGRQLCAPDPQWVERIIQRLQRTSAKMKRSS"
mat_peptide      202..432
                  /gene="ELC"
                  /product="EBI1-ligand chemokine"
polyA_signal      657..662
BASE COUNT       154 a    223 c    173 g    137 t
ORIGIN
    1 cattccagc ctcacatcac tcacaccttg catttcaccc ctgcatccca gtcgccctgc
   61 agcctcacac agatcctgca cacaccaga cagctggcgc tcacacattc accgttggcc
  121 tgctctgtgt caccctccat ggccctgcta ctggccctca gcctgctggt tctctggact
  181 tccccagccc caactctgag tggcaccaat gatgctgaag actgctgccc gtctgtgacc
  241 cagaaaccca tccctgggta catcgtgagg aacttccact accttctcat caaggtggc
  301 tgcagggtgc ctgctgtagt gtccaccaca ctgaggggcc gccagctctg tgcaccccca
  361 gaccagccct gggtagaacg catcatccag agactgcaga ggacctcagc caagatgaag
  421 cgccgcagca gttaacctat gaccgtgcag agggagcccg gagtccgagt caagcattgt
  481 gaattattac ctaacctggg gaaccgagga ccagaaggaa ggaccaggct tccagctcct
  541 ctgcaccaga cctgaccagc caggacaggg cctgggggtgt gtgtgagtgt gagtgtgagc
  601 gagagggtga gtgtggtcag agtaaagctg ctccaccccc agattgcaat gctaccaata
  661 aagccgcctg gtgtttacaa ctaattg

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LOCUS             AB000221             760 bp.    mRNA             PRI             31-JUL-1997
DEFINITION       Homo sapiens mRNA for CC chemokine, complete cds.
ACCESSION        AB000221
NID              g2289718
KEYWORDS         CC chemokine; PARC; pulmonary and activation-regulated
chemokine.
SOURCE           Homo sapiens lung cDNA to mRNA.
ORGANISM         Homo sapiens
                  Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
                  Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae;
                  Homo.
REFERENCE        1 (bases 1 to 760)
AUTHORS          Nomiya, H.
TITLE            Direct Submission
JOURNAL          Submitted (04-JAN-1997) to the DDBJ/EMBL/GenBank databases.
                  Hisayuki Nomiya, Kumamoto University Medical School,
Department       of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan
                  (E-mail:nomiyama@gpo.kumamoto-u.ac.jp, Tel:81-96-373-5063,
                  Fax:81-96-372-6140)
REFERENCE        2 (sites)
AUTHORS          Hieshima, K., Imai, T., Baba, M., Shoudai, K., Ishizuka, K.,
                  Nakagawa, T., Tsuruta, J., Takeya, M., Sakaki, Y., Takatsuki, K.,
                  Miura, R., Opdenakker, G., Damme, J., Yoshie, O. and Nomiya, H.
TITLE            A novel human CC chemokine PARC that is most homologous to
chemotactic      macrophage-inflammatory protein-1alpha/LD78alpha and
                  for T lymphocytes, but not for monocytes
JOURNAL          J. Immunol. 159 (3), 1140-1149 (1997)
MEDLINE          97376836
FEATURES
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    CDS          64..333
                  /gene="PARC"
                  /note="pulmonary and activation-regulated chemokine"

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/codon_start=1
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ORIGIN

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121 tgtgcacaag ttggtaccaa caaagagctc tgctgcctcg tctatacctc ctggcagatt
181 ccacaaaagt tcatagttga ctattctgaa accagcccc agtgcccaa gccagggtgc
241 atcctcctaa ccaagagagg ccggcagatc tgtgctgacc ccaataagaa gtgggtccag
301 aaatacatca gcgacctgaa gctgaatgcc tgaggggacct ggaagctgcg agggcccagt
361 gaacttggtg ggcccaggag ggaacaggag cctgagccag ggcaatggcc ctgccaccct
421 ggagggcacc tcttctaaga gtcccatctg ctatgccag ccacattaac taactttaat
481 cttagtttat gcatcatatt tcattttgaa attgatttct attgttgagc tgcattatga
541 aattagtatt ttctctgaca tctcatgaca ttgtctttat catcctttcc cctttccctt
601 caactcttcg tacattcaat gcatggatca atcagtgatga ttagctttct cagcagacat
661 tgtgccatat gtatcaaatg acaaatcttt attgaatggt tttgctcagc accacctttt
721 aatatattgg cagtacttat tatataaaag gtaaaccagc

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LOCUS      D86955          799 bp      mRNA          PRI          06-MAR-1997
DEFINITION Human mRNA for CC chemokine LARC precursor, complete cds.
ACCESSION  D86955
NID        gl871138
KEYWORDS   CC chemokine LARC precursor.
SOURCE     Homo sapiens cDNA to mRNA.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE  1 (sites)
AUTHORS    Hieshima,K., Imai,T., Opdenakker,G., Van Damme,J., Kusuda,J.,
            Tei,H., Sakaki,Y., Takatsuki,K., Miura,R., Yoshie,O. and
            Nomiya,H.
TITLE      Molecular cloning of a novel human CC chemokine liver and
            activation-regulated chemokine (LARC) expressed in liver.
            Chemotactic activity for lymphocytes and gene localization on
            chromosome 2
JOURNAL    J. Biol. Chem. 272 (9), 5846-5853 (1997)
MEDLINE    97190319
REFERENCE  2 (bases 1 to 799)
AUTHORS    Hieshima,K., Imai,T., Opdenakker,G., Van Damme,J., Kusuda,J.,
            Tei,H., Sakaki,Y., Takatsuki,K., Miura,R., Yoshie,O. and
            Nomiya,H.
JOURNAL    Unpublished (1996)
REFERENCE  3 (bases 1 to 799)
AUTHORS    Nomiya,H.
TITLE      Direct Submission
JOURNAL    Submitted (08-AUG-1996) to the DDBJ/EMBL/GenBank databases.
            Hisayuki Nomiya, Kumamoto University Medical School,
            Department
            of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan
            (E-mail:nomiya@gpo.kumamoto-u.ac.jp, Tel:+81-96-373-5063)
FEATURES   Location/Qualifiers
            source          1..799
                        /organism="Homo sapiens"
                        /db_xref="taxon:9606"
                        /chromosome="2"
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            sig_peptide     59..136
                        /gene="LARC"
            CDS             59..349
                        /gene="LARC"
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mat_peptide   /gene="LARC"
              137..346
              /gene="LARC"
              /product="CC chemokine LARC"
BASE COUNT   240 a   138 c   153 g   268 t
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121 cggcgaatca gaagcagcaa gcaactttga ctgctgtctt ggatacacag accgtattct
181 tcatacctaaa ttattgttgg gcttcacacg gcagctggcc aatgaaggct gtgacatcaa
241 tgctatcatc ttccacacaa agaaaaagtt gtctgtgtgc gcaaatccaa aacagacttg
301 ggtgaaatat attgtgcgtc tcctcagtaa aaaagtcaag aacatgtaaa aactgtggct
361 tttctggaat ggaattggac atagcccaag aacagaaaga accttgctgg ggttggagggt
421 ttcacttgca catcatggag ggtttagtgc ttatctaatt tgtgcctcac tggacttgct
481 caattaatga agttgattca tattgcattc tagtttgctt tgtttaagca tcacattaaa
541 gttaaaactgt attttatggt atttatagct gtaggttttc tgtgttttagc tatttaatac
601 taattttcca taagctattt tggtttagtg caaagtataa aattatattt gggggggaat
661 aagattatat ggactttctt gcaagcaaca agctattttt taaaaaaact atttaacatt
721 cttttgttta tattgttttg tctcctaatt tgttgtaatt gcattataaa ataagaaaaa
781 cattaataag acaaatatt

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LOCUS        HUMAR          538 bp      mRNA          PRI          11-SEP-1996
DEFINITION   Human mRNA for chemokine, complete cds.
ACCESSION    D43767
NID          g1536878
KEYWORDS     chemokine, thymus and activation-regulated; chemokine.
SOURCE       Homo sapiens male peripheral blood cDNA to mRNA, clone:D3A.
ORGANISM     Homo sapiens
              Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
              Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae;
REFERENCE    1 (sites)
AUTHORS      Imai,T., Yoshida,T., Baba,M., Nishimura,M., Kakizaki,M. and
              Yoshie,O.
TITLE        Molecular cloning of a novel T cell-directed CC chemokine
expressed    in thymus by signal sequence trap using Epstein-Barr virus
vector
JOURNAL      J. Biol. Chem. 271 (35), 21514-21521 (1996)
MEDLINE      96355526
REFERENCE    2 (bases 1 to 538)
AUTHORS      Imai,T.
JOURNAL      Unpublished (1996)
REFERENCE    3 (bases 1 to 538)
AUTHORS      Imai,T.
TITLE        Direct Submission
JOURNAL      Submitted (07-DEC-1994) to the DDBJ/EMBL/GenBank databases.
Toshio
              Imai, Shionogi Institute for Medical Science; 2-5-1 Mishima,
              Settsu, Osaka 566, Japan (Tel:06-382-2612, Fax:06-382-2598)
FEATURES
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              /organism="Homo sapiens"
              /db_xref="taxon:9606"
              /clone="D3A"
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  CDS        53..337
              /note="thymus and activation regulated"
              /codon_start=1
              /product="chemokine"
              /db_xref="PID:d1008410"
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/translation="MAPLKMLALVTL LLGASLQHIHAARGTNVGRECCLEYFKGA IPL
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BASE COUNT      118 a      168 c      149 g      103 t
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    1 ccctgagcag agggacctgc acacagagac tccctcctgg gctcctggca ccatggcccc
   61 actgaagatg ctggccctgg tcacctcct cctgggggct tctctgcagc acatccacgc
  121 agctcgaggg accaatgtgg gccgggagtg ctgcctggag tacttcaagg gagccattcc
  181 ccttagaaaag ctgaagacgt ggtaccagac atctgaggac tgctccaggg atgccatcgt
  241 ttttctaact gtgcagggca gggccatctg ttcggacccc aacaacaaga gagtgaagaa
  301 tgcagttaaa tacctgcaaa gccttgagag gtcttgaagc ctctcacc cagactcctg
  361 actgtctccc gggactacct gggacctcca ccgttggtgt tcaccgcccc caccctgagc
  421 gcctgggtcc aggggaggcc ttccagggac gaagaagagc cacagtgagg gagatccat
  481 ccccttgtct gaactggagc catgggcaca aaggggccag attaaagtct ttatcctc

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LOCUS      HUMEOTAXIN      807 bp      mRNA      PRI      25-SEP-1996
DEFINITION Human mRNA for eotaxin, complete cds.
ACCESSION  D49372
NID        g1552240
KEYWORDS   eotaxin; eosinophil-selective CC chemokine; chemoattractant.
SOURCE     Homo sapiens Small intestine, proximal cDNA to mRNA, clone:141.
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE  1 (bases 1 to 807)
AUTHORS   Kitaura,M., Nakajima,T., Imai,T., Harada,S., Combadiere,C.,
            Tiffany,H.L., Murphy,P.M. and Yoshie,O.
TITLE     Molecular cloning of human eotaxin, an eosinophil-selective CC
            chemokine, and identification of a specific eosinophil eotaxin
            receptor, CC chemokine receptor 3
JOURNAL    J. Biol. Chem. 271 (13), 7725-7730 (1996)
MEDLINE    96205964
REFERENCE  2 (bases 1 to 807)
AUTHORS   Yoshie,O.
TITLE     Direct Submission
JOURNAL    Submitted (15-FEB-1995) to the DDBJ/EMBL/GenBank databases.
Osamu
            Yoshie, Shionogi Institute for Medical Science; 2-5-1 Mishima,
            Settsu, Osaka 566, Japan (E-mail:osamu.yoshie@shionogi.co.jp,
            Tel:06-382-2612, Fax:06-382-2598)
COMMENT    On Sep 20, 1996 this sequence version replaced gi:1313900.
FEATURES   Location/Qualifiers
            source          1..807
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                               /db_xref="PID:g1552241"
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            RLESYRRITSGKCPQKAVIFKTKLAKDICADPKKKWVQDSMKYLDQKSPTPKP"
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                               /note="mRNA destabilization signal"
            polyA_signal      775..780
            polyA_site        807

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  121 tgtggctgct gctcatagca gctgccttca gccccaggg gctcgtggg ccagcttctg
  181 tcccaaccac ctgctgcttt aacctggcca ataggaagat accccttcag cgactagaga
  241 gctacaggag aatcaccagt ggcaaatgtc cccagaaagc tgtgatcttc aagaccaaac
  301 tggccaagga tatctgtgcc gaccccaaga agaagtgggt gcaggattcc atgaagtac
  361 tggaccaaaa atctccaact ccaaaagccat aaataatcac catttttgaa accaaaccag
  421 agcctgagtg ttgcctaatt tgttttccct tcttacaatg cattctgagg taacctcatt
  481 atcagtccaa agggcatggg ttttattata tatatatata tttttttttt aaaaaaaac
  541 gtattgcatt taatttatg aggccttaaa acttatcctc catgaatc atgtattttt

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601 aaactgtaaa gctttgtgca gattctttac cccctgggag cccaattcg atccccctgtc
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721 caaagatgat cagtatgaaa atgtcattgt tcttgtgaac ccaaagtgtg actcattaaa
781 tggaagtaaa tgttgtttta ggaatac

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LOCUS       HSCCCHEM       232 bp      RNA              PRI       10-SEP-1996
DEFINITION  H.sapiens mRNA for CC-chemokine.
ACCESSION   Z69291
NID         g1181148
KEYWORDS    CC-chemokine.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1 (bases 1 to 232)
  AUTHORS   Bartels,J.H., Schlueter,C., Richter,E., Christophers,E. and
            Schroeder,J.M.
  TITLE     Cloning of a novel human chemokine homologous to human monocyte
            chemoattractant proteins and rodent eotaxins
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 232)
  AUTHORS   Bartels,J.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-FEB-1996) Bartels J. H.,
            Christian-Albrechts-Universitaet zu Kiel,
            Dermatology/Hautklinik,
            Mol.Biol.Lab.609, Schittenhelmstr. 7, Kiel, Schleswig-Holstein,
            Germany, D-24105
REFERENCE   3 (bases 1 to 232)
  AUTHORS   Bartels,J., Schluter,C., Richter,E., Noso,N., Kulke,R.,
            Christophers,E. and Schroder,J.M.
  TITLE     Human dermal fibroblasts express eotaxin: molecular cloning,
            mRNA
            expression, and identification of eotaxin sequence variants
  JOURNAL   Biochem. Biophys. Res. Commun. 225 (3), 1045-1051 (1996)
  MEDLINE   96374440
FEATURES
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                  /db_xref="taxon:9606"
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                  14(9512),15(9512),10(9601),11(9601)"
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                  /cell_type="fibroblast"
                  /sex="Male"
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                  /citation={1}
                  /product="CC-chemokine"
  sig_peptide     56..109
                  /citation={1}
  CDS             56..>232
                  /function="putative chemoattractant protein"
                  /note="sequence homology to human MCP-1, MCP-2 and
MCP-3            and to rodent eotaxins"
                  /citation={1}
                  /codon_start=1
                  /product="CC-chemokine, preprotein"
                  /db_xref="PID:e221070"
                  /db_xref="PID:g1181149"
                  /db_xref="SWISS-PROT:P50877"
  /translation="MKVSAALLWLLLIAAAFSPQGLAGPASVPTCCFNLANRKIPLQ
                RLESYRRITSGKCPQ"
  mat_peptide     110..>232
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BASE COUNT      55 a      82 c      50 g      42 t      3 others
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        61 ggctccgca gcgcttctgt ggctgctgct catagcggct gccttcagcc cccaggggct
       121 cgctgggcca gcttctgtcc caaccacctg ctgctttaac ctggccaata ggaagatacc
       181 ccttcagcga ctagagagct acaggagaat caccagtggc aaatgtcccc ag

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LOCUS      HSHCC1GEN      4037 bp      DNA              PRI      01-OCT-1995
DEFINITION H.sapiens gene for chemokine HCC-1.
ACCESSION  Z49269
NID        g1004266
KEYWORDS   chemokine.
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 4037)
AUTHORS    Pardigol,A., Maegert,H.J., Cieslak,A., Hill,O., Schulz-
Knappe,P.
TITLE      and Forssmann,W.G.
            Nucleotide Sequence of the Gene for the Human Chemokine HCC-1
JOURNAL    Unpublished
REFERENCE  2 (bases 1 to 4037)
AUTHORS    Pardigol,A.
TITLE      Direct Submission
JOURNAL    Submitted (18-MAY-1995) Andreas Pardigol, Molecular Biology,
Lower
            Saxony Institute for Peptide Research, Feodor-Lynen-Strasse 31,
            Hannover, Lower Saxon, 30625, Germany
FEATURES   Location/Qualifiers
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                           /clone_lib="lambda FIX II, Cat.Nr. 946203, Stratagene"
                           /sex="male"
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            5'UTR           764..833
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rules"
            exon            764..912
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rules;
                           base 780 is the first base of cDNA (Z49270)"
            CDS              join(834..912,3021..3135,3585..3672)
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                           /product="chemokine HCC-1"
                           /db_xref="PID:g1004267"
            /translation="MKISVAAIPFFLLITIALGKTESSSRGPHYHPSECCFTYTTYKI
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            intron          913..3020
                           /number=1
            exon            3021..3135
                           /number=2
            intron          3136..3584
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            exon            3585..3817
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61 agccaaacca cctgaatttg aacccccagt ccatttacca actgtcaaaa gcttaggctt
121 tgattctaag cctgtttcct caactgctgt tctaaagatt aaataggcta atattcataa
181 ggcaactggg acagtggcct gtgtgtatag caaccattat ataagtgaat tatctactga
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301 tgcaggttct gcttcgagtt taagttagga tttcccttga ccaatgagac ctgacttgga
361 ggagtcctgg cctcattcca ttaccccaaa caccctctag tctctagatg aacagatcct
421 gaatgtccag gccccacgtg gcctgttcta aggctgaga tggaattgga tacaggacac

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481 atccagcctt gagatctttt gctaagtgtg acacagtgcc cccagccctg tgctcatgtt
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601 ccatctccctc ccttaaacctt tggctctccc tgcttccttc aggccaccaaa caacacatgt
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3661 aaggagaact gagtaccaca gaaggggtgg cgaaggcaca gctcagagac ataaagagaa
3721 gatgccaagg cccctcctc caccaccgc taactctcag cccagtcac cctctggag
3781 ctctcctgct ttgaattaaa gaccactcat gctcttccct ggctctatc ctttctacgg
3841 gatttactca ttggccatgc actgaggaca ccagggtgtg gcacctcgg catcaagcct
3901 cgctctgcag aagttttggt ggagcctggt acaaaaaata ggtcaggcct gcaatgcagg
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4021 aacagtagca gaccccg

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LOCUS       HSCC21          925 bp      mRNA                      PRI          30-JUN-1998
DEFINITION  H.sapiens mRNA for chemokine CC-2 and CC-1.
ACCESSION  Z70292
NID        g1296608
KEYWORDS    chemokine CC-1; chemokine CC-2.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1 (bases 1 to 925)

```

\* AUTHORS Pardigol, A., Forssmann, U., Zucht, H.D., Loetscher, P.,  
 TITLE Schulz-Knappe, P., Baggiolini, M., Forssmann, W.G. and Magert, H.J.  
 and HCC-2, a human chemokine: gene structure, expression pattern,  
 biological activity  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 95 (11), 6308-6313 (1998)  
 MEDLINE 98263352  
 REFERENCE 2 (bases 1 to 925)  
 AUTHORS Pardigol, A.  
 TITLE Direct Submission  
 JOURNAL Submitted (25-MAR-1996) Andreas Pardigol, IV - Molecular  
 Biology,  
 Lower Saxony Institute for Peptide Research, Feodor-Lynen-  
 Strasse  
 31, Hannover, Lower Saxony, 30625, Germany  
 FEATURES Location/Qualifiers  
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 CDS 56..397  
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 NSFHFADDCCTSYISQSI PCSLMKSYFETSSSECSKPGVIFLTKKGRQVCAKPSGPGVQ  
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 polyA\_signal 902..908  
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 241 cccgtgttca ctcatgaaaa gttattttga aacgagcagc gagtgtctca agccaggtgt  
 301 catattcctc accaagaagg ggcggcaagt ctgtgccaaa cccagtggtc cgggagttca  
 361 ggattgcatg aaaaagctga agccctactc aatataataa taaagagaca aaagaggcca  
 421 gccaccacc tccaacacct cctgagcctc tgaagctccc accaggccag ctctcctccc  
 481 acaacagctt cccacagcat gaagatctcc gtggctgcca ttcctctctt cctcctcatc  
 541 accatcgccc tagggacca gactgaatcc tctcacggg gaccttacca cccctcagag  
 601 tgctgcttca cctacactac ctacaagatc ccgcgtcagc ggattatgga ttactatgag  
 661 accaaccagc agtgctccaa gcccggaatt gtcttcatca ccaaaagggg ccattccgct  
 721 tgtaccaacc ccagtgcaca gtgggtccag gactatatca aggacatgaa ggagaactga  
 781 gtgacccaga aggggtggcg aaggcacagc tcagagacat aaagagaaga tgccaaggcc  
 841 cctcctcca cccaccgcta actctcagcc ccagtcaccc tcttgagct tccctgcttt  
 901 gaattaaaga ccactcatgc tcttc  
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LOCUS HSCC23 973 bp RNA PRI 03-MAY-1996  
 DEFINITION H.sapiens mRNA for chemokine CC-2 and CC-3.  
 ACCESSION Z70293  
 NID g1296611  
 KEYWORDS Human chemokine CC-2; Human chemokine CC-3.  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 973)  
 AUTHORS Pardigol, A., Maegert, H.J., Zucht, H.D., Forssmann, W.G. and  
 Schulz-Knappe, P.  
 TITLE Transcription of a Human Tandem Gene results in a Mature  
 Bicistronic mRNA encoding two Novel CC-Chemokines  
 JOURNAL Unpublished  
 REFERENCE 2 (bases 1 to 973)  
 AUTHORS Pardigol, A.  
 TITLE Direct Submission  
 JOURNAL Submitted (25-MAR-1996) Andreas Pardigol, IV - Molecular  
 Biology,  
 Lower Saxony Institute for Peptide Research, Feodor-Lynen-  
 Strasse  
 31, Hannover, Lower Saxony, 30625, Germany  
 FEATURES  
 source Location/Qualifiers  
 1..973  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 /dev\_stage="adult"  
 /tissue\_type="liver"  
 /clone\_lib="PCR fragments"  
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 CDS 56..397  
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 /db\_xref="PID:g1296612"  
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 misc\_feature 398..498  
 /note="spacing region between two coding regions of  
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 CDS 499..828  
 /note="putative"  
 /codon\_start=1  
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 /db\_xref="PID:g1296613"  
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 IKDMKEN"  
 3'UTR 829..973  
 polyA\_signal 950..956  
 BASE COUNT 257 a 301 c 215 g 200 t  
 ORIGIN  
 1 ccaggaagca gtgagcccag gagtcctcgg ccagccctgc ctgcccacca ggaggatgaa  
 61 ggtctccgtg gctgccctct cctgcctcat gcttggtgct gtccttggat cccaggccca  
 121 gttcacaat gatgcagaga cagagttaat gatgtcaaag cttccactgg aaaatccagt  
 181 agttctgaac agctttcact ttgctgctga ctgctgcacc tcctacatct cacaagcat  
 241 cccgtgttca ctcatgaaaa gttattttga aacgagcagc gaggctcca agccaggtgt  
 301 catattcctc accaagaagg ggcggcaagt ctgtgccaaa cccagtggtc cgggagttca  
 361 ggattgcatg aaaaagctga agccctactc aatataataa taaagagaca aaagaggcca  
 421 gccaccacc tccaacacct cctgagcctc tgaagctccc accaggccag ctctcctccc

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481 acaacagctt cccacagcat gaagatctcc gtggctgcca ttccttctt cctcctcatc
541 accatcgccc tagggaccaa gactgaatcc tcctcacaaa ctggggggaa accgaaggtt
601 gttaaaatac agctaaagtt ggtgggggga ccttaccacc cctcagagtg ctgcttcacc
661 tacactacct acaagatccc gcgtcagcgg attatggatt actatgagac caacagccag
721 tgctccaagc ccggaattgt cttcatcacc aaaagggggc attccgtctg taccaacccc
781 agtgacaagt ggggtccagga ctatatcaag gacatgaagg agaactgagt gaccacagaag
841 ggggtggcgaa ggcacagctc agagacataa agagaagatg ccaaggcccc ctcctccacc
901 caccgctaac tctcagcccc agtcaccctc ttggagcttc cctgctttga attaaagacc
961 actcatgctc ttc

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LOCUS      HSU91746      1430 bp      mRNA      PRI      12-MAR-1998
DEFINITION Homo sapiens IL-10-inducible chemokine (HCC-4) mRNA, complete
cds.
ACCESSION  U91746
NID        g2581780
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 1430)
AUTHORS    Hedrick,J.A., Helms,A., Gorman,D. and Zlotnik,A.
TITLE      Identification of a novel human CC chemokine upregulated by IL-
10
JOURNAL    Blood (1998) In press
REFERENCE  2 (bases 1 to 1430)
AUTHORS    Hedrick,J.A., Helms,A., Gorman,D. and Zlotnik,A.
TITLE      Direct Submission
JOURNAL    Submitted (02-MAR-1997) Immunology, DNAX Research Institute,
901        California Ave, Palo Alto, CA 94304, USA
FEATURES
  source    Location/Qualifiers
            1..1430
            /organism="Homo sapiens"
            /db_xref="taxon:9606"
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            /gene="HCC-4"
  CDS       1..363
            /gene="HCC-4"
            /note="CC or beta chemokine family member"
            /codon_start=1
            /product="IL-10-inducible chemokine"
            /db_xref="PID:g2581781"

/translation="MKVSEAALSLVLILIITSASRSQPKVPEWVNTPTSCCLKYYEK
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TVKIITAKNGQPQLLSQ"
BASE COUNT      401 a      351 c      293 g      385 t
ORIGIN
1 atgaaggtct ccgaggctgc cctgtctctc cttgtctctc tccttatcat tacttcggct
61 tctcgagccc agccaaaagt tcctgagtgg gtgaacaccc catccacctg ctgcctgaag
121 tattatgaga aagtgttgcc aaggagacta gtggtgggat acagaaaggc cctcaactgt
181 cacctgccag caatcatctt cgtaaccaag aggaaccgag aagtctgcac caaccccaat
241 gaggaactgg tccaagagta catcaaggat cccaacctac ctttgcgtgc taccaggaac
301 ttgtccacgg ttaaaattat tacagcaaag aatggtcaac cccagctcct caactcccag
361 tgatgaccag gctttagtgg aagcccttgt ttacagaaga gaggggtaaa cctatgaaaa
421 caggggaagc cttattagtc tgaaactagc cagtcacatt gagagaagca gaacaatgat
481 caaaataaag gagaagtatt tcgaatattt tctcaatctt aggaggaaat accaaagtta
541 agggacgtgg gcagaggtag gctcttttat ttttatattt atatttttat ttttttgaga
601 taggtcttac tctgtcacc aggtctgagt gcagtgggtg gatcttggtc cacttgatct
661 tggctcactg taacctccac ctcccaggct caagtgatcc tcccacccca gcctcccgag
721 tagctgggac tacaggcttg cgccaccaca cctggctaatt ttttgtattt ttggtagaga
781 cgggattcta ccatgttgcc caggctggtc tcaaactcgt gtgccaagc aatccacctg
841 cctcagcctt ccaaaaagtgc tgggattaca ggcgtgagcc accacatccg gccagtgac
901 tcttaataca cagaaaaata tatttcacat ccttctctg ctctctttca attcctcact
961 tcacaccagt acacaagcca ttctaataac ttagccagtt tccagccttc cagatgatct
1021 ttgccctctg ggtcttgacc cattaagagc cccatagaac tcttgatttt tcctgtccat
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1141 tttttcatag gaagtcgga tgggaatatt cacattaatc atttttgcag agactttgct
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1261 aaaaaaaaaa aaagagagag agagagaaga agaagaagaa gagacacaaa tctctacctc
1321 ccatgttaag ctttcagga cagggaaaga aagggtatga gacacggcta ggggtaaact
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LOCUS      AB007454      1503 bp      mRNA                      PRI      09-APR-1998
DEFINITION Homo sapiens mRNA for chemokine LEC precursor, complete cds.
ACCESSION  AB007454
NID        g2723285
KEYWORDS   chemokine LEC precursor.
SOURCE     Homo sapiens liver cDNA to mRNA.
ORGANISM   Homo sapiens
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (sites)
AUTHORS    Shoudai,K., Hieshima,K., Fukuda,S., Iio,M., Miura,R., Imai,T.,
            Yoshie,O. and Nomiyama,H.
TITLE      Isolation of cDNA encoding a novel human CC chemokine NCC-4/LEC
JOURNAL    Biochim. Biophys. Acta 1396 (3), 273-277 (1998)
MEDLINE    98207719
REFERENCE  2 (bases 1 to 1503)
AUTHORS    Nomiyama,H.
TITLE      Direct Submission
JOURNAL    Submitted (19-SEP-1997) to the DDBJ/EMBL/GenBank databases.
            Hisayuki Nomiyama, Kumamoto University Medical School,
Department of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860-0811,
Japan
            (E-mail:nomiyama@gpo.kumamoto-u.ac.jp, Tel:81-96-373-5063,
            Fax:81-96-372-6140)

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FEATURES             Location/Qualifiers
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                       /tissue_type="liver"
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     CDS               77..439
                       /codon_start=1
                       /product="chemokine LEC precursor"
                       /db_xref="PID:d1024963"
                       /db_xref="PID:g2723286"

/translation="MKVSEALSLVLILITSASRSQPKVPEWNTPTSCCLKYYEK
VLPRLVVG YRKALNCHLPAIIFVTKRNRVCTNPNDWVQEYIKDPNLPLLPTRNLS
TVKIITAKNGQPQLNSQ"
     mat_peptide       146..436
     polyA_signal      560..565
     polyA_signal      1485..1490
BASE COUNT    417 a      374 c      312 g      400 t
ORIGIN
1 gttggcaagc ggaccaccag caacagacaa catcttcatt cggctctccc tgaagctgta
61 ctgcctcgct gagaggatga aggtctccga ggctgccttg tctctccttg tctcatcctt
121 tatcattact tcggcttctc gcagccagcc aaaagttcct gagtgggtga acaccccatc
181 cactgtgctg ctgaagtatt atgagaaagt gttgccaagg agactagtgg tgggatacag
241 aaaggccctc aactgtcacc tgccagcaat catcttcgtc accaagagga accgagaagt
301 ctgcaccaac cccaatgacg actgggtcca agagtacatc aaggatccca acctaccttt
361 gctgcctacc aggaacttgt ccacggttaa aattattaca gcaaagaatg gtcaacccca
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721 cttggctcac ttgatcttgg ctactgttaa cctccacctc ccagggtcaa gtgatcctcc
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841 tgtatttttg gtagagacgg gattctacca tgttgcccag gctgggtctc aactcgtgtg
901 cccaagcaat ccacctgcct cagccttcca aaagtgcctg gattacagc gtgagccacc
961 acatccggcc agtgcactct taatacacag aaaaaatata ttcacatcct tctcctgtct
1021 tctttcaatt cctcacttca caccagtaca caagccattc taaatactta gccagtttcc

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1321 gagatgtcct gattggaaaa aaaaaaaaaa gagagagaga gagaagaaga agaagaagag
1381 acacaaatct ctacctccca tgttaagctt tgcaggacag ggaagaaag ggtatgagac
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1501 ttg

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LOCUS      AF001979      800 bp      mRNA      PRI      20-NOV-1997
DEFINITION Homo sapiens beta chemokine mRNA, complete cds.
ACCESSION  AF001979
NID        g2624924
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 800)
AUTHORS    Hedrick, J.A. and Zlotnik, A.
TITLE      Identification and characterization of a novel beta chemokine
            containing six conserved cysteines
JOURNAL     J. Immunol. 159 (4), 1589-1593 (1997)
MEDLINE     97400322
REFERENCE  2 (bases 1 to 800)
AUTHORS    Hedrick, J.A. and Zlotnik, A.
TITLE      Direct Submission
JOURNAL     Submitted (01-MAY-1997) Immunobiology, DNAX Research Institute,
901

```

California Ave, Palo Alto, CA 94304, USA

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FEATURES             Location/Qualifiers
     source            1..800
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                       /db_xref="taxon:9606"
     CDS                1..405
                       /note="6Ckine; CC chemokine"
                       /codon_start=1
                       /product="beta chemokine"
                       /db_xref="PID:g2624925"

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BASE COUNT      203 a      248 c      210 g      139 t
ORIGIN

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1 atggctcagt cactggctct gagcctcctt atcctgggtc tggcctttgg aatccccagg
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121 ccgcgaagg ttgtccgag ctaccggaag caggaacca gcttaggctg ctccatccca
181 gctatcctgt tcttgccccg caagcgctct caggcagagc tatgtgcaga cccaaaggag
241 ctctgggtgc agcagctgat gcagcatctg gacaagacac catccccaca gaaaccagcc
301 cagggctgca ggaaggacag gggggcctcc aagactggca agaaaggaaa gggctccaaa
361 ggctgcaaga ggactgagcg gtcacagacc cctaaagggc catagcccag tgagcagcct
421 gagccctgg agacccacc agcttcacca gcgcttgaag cctgaacca agatgcaaga
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541 ctctgcttt aaccaccca tctgcattcc cagctctacc ctgcatggct gagctgcccc
601 cagcaggcca ggtccagaga gaccgaggag ggagagtctc ccaggagga tgagaggagg
661 cagcaggact gtcccttga aggagaatca tcaggaccct ggacctgata cggctcccca
721 gtacaccca cctcttctt gtaaataatga ttataccta actgaataaa aagctgttct
781 gtcttccac ccaaaaaaa

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LOCUS      HSU64197      821 bp      mRNA      PRI      25-JUN-1997
DEFINITION Homo sapiens chemokine exodus-1 mRNA, complete cds.
ACCESSION  U64197
NID        g1778716
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;

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Hominidae; Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;  
 Homo.  
 REFERENCE 1 (bases 1 to 821)  
 AUTHORS Hromas, R., Gray, P.W., Chantry, D., Godiska, R., Krathwohl, M.,  
 Fife, K., Bell, G.I., Takeda, J., Aronica, S., Gordon, M.,  
 Cooper, S., Broxmeyer, H.E. and Klemsz, M.J.  
 TITLE Cloning and characterization of exodus, a novel beta-chemokine  
 JOURNAL Blood 89 (9), 3315-3322 (1997)  
 MEDLINE 97275143  
 REFERENCE 2 (bases 1 to 821)  
 AUTHORS Hromas, R.A.  
 TITLE Direct Submission  
 JOURNAL Submitted (17-JUL-1996) Indiana University Medical Center,  
 Medicine, 975 W. Walnut St., Indianapolis, IN 46202, USA  
 FEATURES Location/Qualifiers  
 source 1..821  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 /clone="Exodus-1"  
 /cell\_type="islet"  
 /tissue\_type="pancreas"  
 /dev\_stage="adult"  
 CDS 43..330  
 /function="inhibits proliferation of hematopoietic  
 progenitors and HIV"  
 /codon\_start=1  
 /product="chemokine exodus-1"  
 /db\_xref="PID:g177871"  
 /translation="MCCTKSLLLAALMSVLLLHLCESEASNFDCCCLGYTDRILHPKF  
 IVGFTRQLANEGCDINAIIFHTKKLSVCANPKQTWVKYIVRLLSKKVKNM"  
 variation 121^122  
 /note="insertion of an extra codon GCA at nt 121,  
 encoding for an alanine after the alanine at amino acid  
 position 26, represents the allelic difference of the  
 transcript isolated from macrophages"  
 BASE COUNT 258 a 134 c 156 g 273 t  
 ORIGIN  
 1 ggtactcaac actgagcaga tctgttcttt gagctaaaaa ccatgtgctg taccaagagt  
 61 ttgctcctgg ctgctttgat gtcagtgtcg ctactccacc tctgcggcga atcagaagca  
 121 agcaactttg actgctgtct tggatacaca gaccgtattc ttcatcctaa atttattgtg  
 181 ggcttcacac ggcagctggc caatgaaggc tgtgacatca atgctatcat ctttcacaca  
 241 aagaaaaagt tgtctgtgtg cgcaaatcca aaacagactt gggtgaaata tattgtgcgt  
 301 ctctcagta aaaaagtcaa gaacatgtaa aaactgtggc ttttctggaa tggaattgga  
 361 catagcccaa gaacagaaag aaccttgctg gggttggagg ttacacttgc acatcatgga  
 421 gggtttagtg cttatctaat ttgtgcctca cctggacttg tccaattaat gaagttgatt  
 481 catattgcat catagtttgc tttgtttaag catcacatta aagtgaact gtattttatg  
 541 ttatttatag ctgtaggttt tctgtgttta gctatttaac actaattttc cataagctat  
 601 tttgggttag tgcaagatat aaaattatat ttggggggga ataagattat atggactttc  
 661 ttgcaagcaa caagctattt tttaaaaaaa actatttaac attcctttgt ttatattgtt  
 721 ttgtctccta aattgttgta atgtcattat aaaataagaa aaatattaat aagacaaata  
 781 ttgaaaataa agaaacaaaa agtgcttctg ttaaaaaaaa a  
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LOCUS HSU88320 828 bp mRNA PRI 18-DEC-1997  
 DEFINITION Human beta chemokine Exodus-2 mRNA, complete cds.  
 ACCESSION U88320  
 NID g2196919  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;  
 Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 828)  
 AUTHORS Hromas, R., Kim, C.H., Klemsz, M., Krathwohl, M., Fife, K.,  
 Cooper, S.,

Schnizlein-Bick, C. and Broxmeyer, H.E.  
 TITLE Isolation and characterization of Exodus-2, a novel C-C  
 chemokine  
 JOURNAL with a unique 37-amino acid carboxyl-terminal extension  
 MEDLINE J. Immunol. 159 (6), 2554-2558 (1997)  
 REFERENCE 97444139  
 2 (bases 1 to 828)  
 AUTHORS Hromas, R.A.  
 TITLE Direct Submission.  
 JOURNAL Submitted (04-FEB-1997) Medicine, Indiana University Medical  
 Center, 975 West Walnut, Indianapolis, IN 46202, USA  
 FEATURES Location/Qualifiers  
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 /db\_xref="taxon:9606"  
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 /cell\_type="monoblast"  
 CDS 15..419  
 /codon\_start=1  
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 /db\_xref="PID:g2196920"  
 /translation="MAQSLALSLILVLAFGIPRTQGS DGAQDCCLKYSQRKIPAKV  
 VRSYRKQEPSLGC SIPAILFLPRKRSQAELCADPKELWVQQLMQHLDKTPSPQKPAQG  
 CRKDRGASKTGKKGKSGKCKRTSQT PKGP"  
 BASE COUNT 218 a 255 c 216 g 139 t  
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 1 ggcacgagggc agacatggct cagtcaactgg ctctgagcct ccttatcctg gttctggcct  
 61 ttggcatccc caggacccaa ggcagtgatg gaggggctca ggactgttgc ctcaagtaca  
 121 gccaaaggaa gattcccgcc aaggttgctc gcagctaccg gaagcaggaa ccaagcttag  
 181 gctgctccat ccagctatc ctgttcttgc cccgcaagcg ctctcaggca gagctatgtg  
 241 cagacccaaa ggagctcttg gtgcagcagc tgatgcagca tctggacaag acaccatccc  
 301 cacagaaaacc agcccagggc tgcaggaagg acaggggggc ctccaagact ggcaagaaag  
 361 gaaagggctc caaaggctgc aagaggactg agcggtcaca gaccctctaa gggccatagc  
 421 ccagtgaagc gcctggagcc ctggagaccc caccagcctc accagcgctt gaagcctgaa  
 481 cccaagatgc aagaaggagg ctatgctcag gggccctgga gcagccaccc catgctggcc  
 541 ttgccacact ctttctcctg ctttaaccac cccatctgca tcccagctc tcacctgca  
 601 tggctgagtc tgcccacagc aggccagggtc cagagagacc gaggagggag agtctcccag  
 661 ggagcatgag aggaggcagc aggactgtcc ccttgaagga gaatcatcag gaccctggac  
 721 ctgatacggc tccccagtac accccacctc ttccttgtaa atatgattta tacctaactg  
 781 aataaaaagc tgttctgtct tcccacccaa aaaaaaaaaa aaaaaaaaaa  
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 LOCUS HSU88321 502 bp mRNA PRI 22-JUN-1998  
 DEFINITION Human beta chemokine Exodus-3 mRNA, complete cds.  
 ACCESSION U88321  
 NID g2196921  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;  
 Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 502)  
 AUTHORS Hromas, R.A., Gray, P., Klemsz, M., Fife, K. and Broxmeyer, H.  
 TITLE DCCL chemokines represent a novel beta chemokine subfamily  
 JOURNAL Unpublished  
 REFERENCE 2 (bases 1 to 502)  
 AUTHORS Hromas, R.A.  
 TITLE Direct Submission  
 JOURNAL Submitted (04-FEB-1997) Medicine, Indiana University Medical  
 Center, 975 West Walnut, Indianapolis, IN 46202, USA  
 REFERENCE 3 (bases 1 to 502)  
 AUTHORS Hromas, R.A.  
 TITLE Direct Submission  
 JOURNAL Submitted (22-JUN-1998) Medicine, Indiana University Medical  
 Center, 975 West Walnut, Indianapolis, IN 46202, USA  
 REMARK Amino acid sequence updated by submitter  
 FEATURES Location/Qualifiers  
 source 1..502

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/organism="Homo sapiens"
/note="PCR amplified from THP-1 cells"
/db_xref="taxon:9606"
/cell_line="THP-1"
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/dev_stage="adult"
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120..416
/note="Mip-3alpha/ELC/CKbeta11"
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/product="beta chemokine Exodus-3"
/db_xref="PID:g3243080"

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/translation="MALLLALSLLVLWTSPTLSGTNDAEDCCLSVTQKPIPGYIVR

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BASE COUNT      113 a      170 c      121 g      98 t
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121 tgccctgct actggccctc agcctgctgg ttctctggac tccccagcc ccaactctga
181 gtggcaccaa tgatgctgaa gactgctgcc tgtctgtgac ccagaaaccc atccctgggt
241 acatcgtgag gaacttcacc taccttctca tcaaggatgg ctgcagggtg cctgctgtag
301 tgttcaccac actgaggggc cgccagctct gtgcaccccc agaccagccc tgggtagaac
361 gcacatcca gagactgcag aggacctcag ccaagatgaa gcgccgcagc agttaacctt
421 tgaccgtgca gagggagccc cgagtccgag tcaagcattg tgaattatta ctaactggga
481 acgaggacag aaggaaggac ag

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LOCUS      HSU86358      879 bp      mRNA      PRI      11-SEP-1997
DEFINITION Human chemokine (TECK) mRNA, complete cds.
ACCESSION  U86358
NID        g2388626
KEYWORDS
SOURCE      human.
ORGANISM    Homo sapiens
            Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1 (bases 1 to 879)
AUTHORS     Vicari,A.P., Figueroa,D.J., Hedrick,J.A., Foster,J.S.,
Singh,K.P., Menon,S., Copeland,N.G., Gilbert,D.J., Jenkins,N.A., Bacon,K.B.
and         Zlotnik,A.
TITLE       TECK: a novel cc chemokine specifically expressed by thymic
            dendritic cells and potentially involved in T cell development
JOURNAL     Immunology 7, 291-301 (1997)
REFERENCE   2 (bases 1 to 879)
AUTHORS     Vicari,A.P. and Zlotnik,A.
TITLE       Direct Submission
JOURNAL     Submitted (21-JAN-1997) Immunology, DNAX Research Institute,
901         California Ave., Palo Alto, CA 94304, USA
FEATURES    Location/Qualifiers
            source          1..879
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            gene            1..879
                        /gene="TECK"
            CDS              1..453
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                        /codon_start=1
                        /product="chemokine"
                        /db_xref="PID:g2388627"
/translation="MNLWLLACLVLGAWAPAVHTQGVFEDCCLAYHYPIGWAVLR
RAWTYRIQEVSGSCNLPAAIFYLPKRHRKVCGNPKSREVQAMKLLDARNKVFAKLHH
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BASE COUNT      191 a      264 c      218 g      206 t

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## ORIGIN

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1 atgaacctgt ggctcctggc ctgcctgggt gccggcttcc tgggagcctg ggcccccgct
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121 gctgtgctcc ggcgcgcctg gacttaccgg atccaggagg tgagcgggag ctgcaatctg
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241 agggaggtgc agagagccat gaagctcctg gatgctcgaa ataaggtttt tgcaaaagctc
301 caccacaaca tgcagacctt ccaagcaggc cctcatgctg taaagaagtt gagttctgga
361 aactccaagt tatcatcatc caagttagc aatcccatca gcagcagcaa gaggaatgtc
421 tccctcctga tatcagctaa ttcaggactg tgagccggct catttctggg ctccatcggc
481 acaggagggg ccggatcttt ctccgataaa accgtcgccc tacagacca gctgtcccca
541 cgctctgtc ttttgggtca agtcttaatc cctgcacctg agttggctct cctctgcac
601 cccaccacc tcctgcccgt ctggcaactg gaaagaagga gttggcctga ttttaacctt
661 ttgccgctcc gggaacagc acaatcctg gcagccagt gctcttgtag agaaaactta
721 ggatacctct ctactttct gttcttgcc gtccaccccg ggccatgcca gtgtgtcctc
781 tgggtccctt ccaaaaatct ggtcattcaa ggatccctc ccaaggctat gcttttctat
841 aactttttaa taaaccttgg ggggtgaatg gaataaaaa

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LOCUS AB002409 852 bp mRNA PRI 15-AUG-1997

DEFINITION Homo sapiens mRNA for SLC, complete cds.

ACCESSION AB002409

NID g2335034

KEYWORDS SLC; mature ELC.

SOURCE Homo sapiens cDNA to mRNA.

ORGANISM Homo sapiens  
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;  
Hominidae;  
Homo.

REFERENCE 1 (bases 1 to 852)

AUTHORS Nomiyama, H.

TITLE Direct Submission

JOURNAL Submitted (28-MAR-1997) to the DDBJ/EMBL/GenBank databases.  
Hisayuki Nomiyama, Kumamoto University Medical School,  
Department of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan  
(E-mail: nomiyama@gpo.kumamoto-u.ac.jp, Tel: 81-96-373-5063,  
Fax: 81-96-372-6140)

REFERENCE 2 (bases 1 to 852)

AUTHORS Nagira, M., Imai, T., Hieshima, K., Kusuda, J., Ridanpaa, M.,  
Takagi, S., Nishimura, M., Kakizaki, M., Nomiyama, H. and Yoshie, O.

TITLE Molecular Cloning of a Novel Human CC Chemokine Secondary  
Lymphoid-Tissue Chemokine (SLC) That is an Efficient  
Chemoattractant for Lymphocytes and Mapped to Chromosome 9p13

JOURNAL Unpublished (1997)

FEATURES

source Location/Qualifiers

1..852

/organism="Homo sapiens"

/db\_xref="taxon:9606"

CDS 59..463

/codon\_start=1

/product="SLC"

/db\_xref="PID:d1022673"

/db\_xref="PID:g2335035"

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CRKDRGASKTGKKGKSGCKRTSQTPKGK

mat\_peptide <107..460

/product="mature ELC"

polyA\_site 823..828

BASE COUNT 205 a 279 c 217 g 151 t

ORIGIN

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121 ccaaggcagt gatggagggg ctcaggactg ttgcctcaag tacagccaaa ggaagattcc
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241 tatcctgttc ttgccccgca agcgctctca ggagagcta tgtgcagacc caaaggagct
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361 gggctgcagg aaggacaggg gggcctccaa gactggcaag aaaggaaagg gctccaaagg
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481 agccctggag accccaccag cctcaccaac gcttgaagcc tgaacccaag atgcaagaag
541 gaggtatgc tcagggggccc tggagcagcc accccatgct ggccttgcca cactctttct
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661 gcaggccagg tccagagaga ccgaggagg agagtctccc agggagcatg agaggaggca
721 gcaggactgt ccccttgaag gagaatcatc aggaccctgg acctgatacg gctccccagt
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841 cttccccacc gc

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LOCUS      AF055467      1481 bp      mRNA                      PRI      06-AUG-1998
DEFINITION Homo sapiens monotactin-1 mRNA, complete cds.
ACCESSION  AF055467
NID        g3395775
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 1481)
AUTHORS    Youn,B.S., Zhang,S., Broxmeyer,H.E., Antol,K., Fraser,M.J. Jr.,
            Hangoc,G. and Kwon,B.S.
TITLE      Isolation and characterization of LMC, a novel lymphocyte and
            monocyte chemoattractant human CC chemokine, with
            myelosuppressive
            activity
JOURNAL     Biochem. Biophys. Res. Commun. 247 (2), 217-222 (1998)
MEDLINE     98308096
REFERENCE   2 (bases 1 to 1481)
AUTHORS     Youn,B.S. and Kwon,B.S.
TITLE       Direct Submission
JOURNAL     Submitted (24-MAR-1998) Microbiology and Immunology, Indiana
            University, School of Medicine, 605 Barnhill Dr. Medical
            Science
            Bldg., Indianapolis, IN 46202, USA
FEATURES    Location/Qualifiers
            source          1..1481
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                        /chromosome="17"
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            CDS             35..397
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                        /codon_start=1
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                        /db_xref="PID:g3395776"

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398..1481

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3'UTR
BASE COUNT      412 a      362 c      302 g      405 t
ORIGIN

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121 gtgggtgaac accccatcca cctgctgcct gaagtattat gagaagtggt tgccaaggag
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241 caagaggaac cgagaagtct gcaccaaccc caatgacgac tgggtccaag agtacatcaa
301 ggatcccaac ctacctttgc tgcctaccag gaacttgctc acggttaaaa ttattacagc
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421 ttgtttacag aagagagggg taaactatga aaacagggga agccttatta ggctgaaact
481 agccagtcac attgagagaa gcagaacaat gatcaaaata aaggagaagt atttcgaata
541 ttttctcaat cttaggagga aataccaaag ttaagggacg tgggcagagg tacgtctttt
601 tatttttata tttatatatt tatttttttg agatagggtc ttactctgtc acccaggtgt
661 gagtgcagtg gtgtgatctt ggctcacttg atcttggtc actgtaacct ccacctccca
721 ggctcaagtg atcctccca cccaccctcc cgagttagct ggactacagg cttgcgccac
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LOCUS      HSRNAATAC      557 bp      RNA      PRI      06-JUL-1995
DEFINITION H.sapiens mRNA for ATAC protein.
ACCESSION  X86474
NID        g895846
KEYWORDS   ATAC gene.
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 557)
AUTHORS    Muller,S., Dorner,B., Korthauer,U., Mages,H.W., D'Apuzzo,M.,
            Senger,G. and KroczeK,R.A.
TITLE      Cloning of ATAC, an activation-induced, chemokine-related
molecule  exclusively expressed in CD8+ T lymphocytes
JOURNAL     Eur. J. Immunol. 25 (6), 1744-1748 (1995)
MEDLINE     95339892
REFERENCE   2 (bases 1 to 557)
AUTHORS     KroczeK,R.A.
TITLE       Direct Submission
JOURNAL     Submitted (20-APR-1995) R.A. KroczeK, Molecular Immunology,
            Robert-Koch-Institute, Nordufer 20, 13353 Berlin, FRG

FEATURES   Location/Qualifiers
            source      1..557
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            gene        25..369
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            CDS          25..369
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                        /db_xref="SWISS-PROT:P47992"

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IKTYTITEGSLRAVIFITKRGKVCADPQATWVRDVRSMDRKSNTNRNMIQT KPTGT
QQSTNTAVTLTG"
polyA_signal 469..474
polyA_signal 534..539
BASE COUNT  157 a   139 c   112 g   149 t
ORIGIN
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61 tctctcactg catacattgt ggaaggtgta gggagtgaag tctcagataa gaggacctgt
121 gtgagcctca ctaccacagc actgccggtt agcagaatca agacctacac catcacggaa
181 ggctccttga gagcagtaat ttttattacc aaacgtggcc taaaagtctg tgctgatcca
241 caagccacat ggggtgagaga cgtggtcagg agcatggaca ggaaatccaa caccagaaat
301 aacatgatcc agaccaagcc aacaggaacc cagcaatcga ccaatacagc tgtgactctg
361 actggctagt agtctctggc accctgtccg tctccagcca gccagctcat ttcactttac
421 acgctcatgg actgagttta tactcgctt ttatgaaagc actgcatgaa taaaattatt
481 cctttgtatt tttactttta aatgtcttct gtattcactt atatgttcta attaataaat
541 tatttattat taagaat

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LOCUS HSU85767 563 bp mRNA PRI 01-APR-1997  
 DEFINITION Human myeloid progenitor inhibitory factor-1 MPIF-1 mRNA,  
 complete  
 cds.  
 ACCESSION U85767  
 NID g1916249  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 563)  
 AUTHORS Patel,V.P., Kreider,B.L., Li,Y., Li,H., Leung,K., Salcedo,T.,  
 Nardelli,B., Pippalla,V., Gentz,S., Thotakura,R., Parmelee,D.,  
 Gentz,R. and Garotta,G.  
 TITLE Molecular and functional characterization of two novel human C-  
 chemokines as inhibitors of two distinct classes of myeloid  
 progenitors  
 J. Exp. Med. (1997) In press  
 REFERENCE 2 (bases 1 to 563)  
 AUTHORS Li,H. and Patel,V.P.  
 TITLE Direct Submission  
 JOURNAL Submitted (17-JAN-1997) Cell Biology, Human Genome Sciences,  
 9410  
 Keywest Ave., Rockville, MD 20850, USA  
 FEATURES Location/Qualifiers  
 source 1..563  
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 /db\_xref="taxon:9606"  
 CDS 31..393  
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 /db\_xref="PID:g1916250"  
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 BASE COUNT 164 a 143 c 117 g 139 t  
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 1 ctcagccagc cctgcctgcc caccaggagg atgaaggctc ccgtggctgc cctctcctgc  
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 181 agtgctgact gctgcatctc ctacaccca cgaagcatcc cgtgttcact cctggagagt  
 241 tactttgaaa cgaacagcga gtgctccaag ccgggtgtca tcttctcac caagaagggg  
 301 cgacgtttct gtgccaaccc cagtataag caagttcagg ttgcatgag aatgctgaag  
 361 ctggacacac ggatcaagac caggaagaat tgaacttgtc aaggtgaagg gacacaagtt  
 421 gccagccacc aactttcttg cctcaactac cttcctgaat tttttttta agaagcattt  
 481 attcttgtgt tctggattta gagcaattca tctaataaat agtttctcac ttttaaaaaa  
 541 aaaaaaaaaa aaaaaaaaaa aaa  
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LOCUS HSU85768 360 bp mRNA PRI 01-APR-1997  
 DEFINITION Human myeloid progenitor inhibitory factor-1 MPIF-2 mRNA,  
 complete  
 cds.  
 ACCESSION U85768  
 NID g1916251  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 360)  
 AUTHORS Patel,V.P., Kreider,B.L., Li,Y., Li,H., Leung,K., Salcedo,T.,  
 Nardelli,B., Pippalla,V., Gentz,S., Thotakura,R., Parmelee,D.,  
 Gentz,R. and Garotta,G.  
 TITLE Molecular and functional characterization of two novel human C-

C

chemokines as inhibitors of two distinct classes of myeloid progenitors

J. Exp. Med. (1997) In press

JOURNAL REFERENCE 2 (bases 1 to 360)

AUTHORS Li, H. and Patel, V.P.

TITLE Direct Submission

JOURNAL Submitted (17-JAN-1997) Cell Biology, Human Genome Sciences, 9410

Keywest Ave., Rockville, MD 20850, USA

FEATURES Location/Qualifiers

source 1..360

/organism="Homo sapiens"

/db\_xref="taxon:9606"

CDS 1..360

/note="myeloid progenitor inhibitory factor-2"

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VAVKGPVQRYPGNQTTTC"

BASE COUNT 85 a 106 c 96 g 73 t

ORIGIN

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121 agaattcctg agaaccgagt ggtcagctac cagctgtcca gcaggagcac atgcctcaag

181 ggaggagtga tcttcaccac caagaaggc cagcagttct gtggcgaccc caagcaggag

241 tgggtccaga ggtacatgaa gaacctggac gccaaagcaga agaaggcttc ccctagggcc

301 agggcagtggt ctgtcaaggg ccctgtccag agatatcctg gcaaccaaac cacctgctaa

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LOCUS HUMSDF1A 1847 bp mRNA PRI 26-DEC-1996

DEFINITION Human pre-B cell stimulating factor homologue (SDF1a) mRNA, complete cds.

ACCESSION L36034

NID g1220363

KEYWORDS intercrine; intercrine CXC subfamily; pre-B cell stimulating factor

homologue; alpha-chemokine.

SOURCE human.

ORGANISM Homo sapiens

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 1847)

AUTHORS Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinohara, T. and Honjo, T.

TITLE Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene

JOURNAL Genomics 28 (3), 495-500 (1995)

MEDLINE 96039262

FEATURES Location/Qualifiers

source 1..1847

/organism="Homo sapiens"

/db\_xref="taxon:9606"

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/cell\_line="FLEB14-14"

sig\_peptide 80..142

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CDS 80..349

/codon\_start=1

/product="pre-B cell stimulating factor homologue"

/db\_xref="PID:g1220364"

/translation="MNAKVVVVLVLVTALCLSDGKPVLSYRCPFRFFESHVARANV

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/gene="SDF1a"

mat\_peptide 143..346

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/ gene="SDF1a"
/ product="pre-B cell stimulating factor homologue"
BASE COUNT      459 a      471 c      417 g      500 t
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121 cgcgctctgc ctcagcgacg ggaagcccgt cagcctgagc tacagatgcc catgccgatt
181 cttcgaaagc catgttgcca gagccaaagt caagcatctc aaaattctca acactccaaa
241 ctgtgccctt cagattgtag ccgggtgaa gaacaacaac agacaagtgt gcattgaccc
301 gaagctaaag tggattcagg agtacctgga gaaagcttta aacaagtaag cacaacagcc
361 aaaaaggact ttccgctaga cccactcgag gaaaactaaa accttgtgag agatgaaagg
421 gcaaagacgt gggggagggg gccttaacca tgaggaccag gtgtgtgtgt ggggtgggca
481 cattgatctg ggatcggggc tgagggttgc agcatttaga ccctgcattt atagcatacg
541 gtatgatatt gcagcttata ttcatccatg ccctgtacct gtgcacgttg gaacttttat
601 tactggggtt tttctaagaa agaaattgta ttatcaacag cattttcaag cagttagtcc
661 cttcatgatc atcacaaatc tcatcattct cattctcatt ttttaaatca acgagtactt
721 caagatctga atttggcttg tttggagcat ctctctgctt cccctgggga gtctgggcac
781 agtcagggtg tggcttaaca gggagctgga aaaagtgtcc tttcttcaga cactgaggct
841 cccgcagcag cgcccctccc aagagggaagg cctctgtggc actcagatac cgactggggc
901 tggggcgccg ccactgcctt cactcctctt tcaaacctc agtgattggc tctgtgggct
961 ccatgtagaa gccactatta ctgggactgt ctccagagacc cctctcccag ctattcctac
1021 tctctcccgc actccgagag catgcttaat cttgcttctg cttctcattt ctgtagcctg
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ACCESSION  L36033
NID        g1220365
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SOURCE     homologue; alpha-chemokine.
ORGANISM   human.
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REFERENCE  1 (bases 1 to 3524)
AUTHORS    Shirozu,M., Nakano,T., Inazawa,J., Tashiro,K., Tada,H.,
            Shinohara,T. and Honjo,T.
TITLE      Structure and chromosomal localization of the human stromal
            cell-derived factor 1 (SDF1) gene
JOURNAL     Genomics 28 (3), 495-500 (1995)
MEDLINE    96039262
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DEFINITION Homo sapiens cDNA for a CXC chemokine.
ACCESSION  AJ002211

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NID g2832410  
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 REFERENCE 1 (bases 1 to 663)  
 AUTHORS Legler, D.F., Loetscher, M., Roos, R.S., Clark-Lewis, I.,  
 Baggiolini, M.  
 and Moser, B.  
 TITLE B cell-attracting chemokine 1, a human CXC chemokine expressed  
 in lymphoid tissues, selectively attracts B lymphocytes via  
 BLR1/CXCR5  
 JOURNAL J. Exp. Med. 187 (4), 655-660 (1998)  
 MEDLINE 98130629  
 REFERENCE 2 (bases 1 to 663)  
 AUTHORS Moser, B.  
 TITLE Direct Submission  
 JOURNAL Submitted (05-NOV-1997) Moser B., University of Bern, Theodor  
 Kocher Institute, Freiestrasse 1, CH-3012 Bern, SWITZERLAND  
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 REFERENCE 1 (bases 1 to 2545)  
 AUTHORS Farber, J.M.  
 TITLE Direct Submission  
 JOURNAL Submitted (22-MAR-1993) J.M. Farber, Johns Hopkins Univ. School

of  
 USA  
 Medicine, Ross 1147, 720 Rutland Avenue, Baltimore, MD 21205,  
 USA  
 REFERENCE 2 (bases 1 to 2545)  
 AUTHORS Farber, J.M.  
 TITLE HuMig: a new human member of the chemokine family of cytokines  
 JOURNAL Biochem. Biophys. Res. Commun. 192 (1), 223-230 (1993)  
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DEFINITION H.sapiens Humig mRNA.
ACCESSION  X72755 S60728
NID        g311375
KEYWORDS   chemokine; cytokine; Humig gene; secreted protein.
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 2545)
AUTHORS    Farber, J.M.
TITLE      Direct Submission
JOURNAL    Submitted (22-MAR-1993) J.M. Farber, Johns Hopkins Univ. School
of         Medicine, Ross 1147, 720 Rutland Avenue, Baltimore, MD 21205,
USA
REFERENCE  2 (bases 1 to 2545)
AUTHORS    Farber, J.M.
TITLE      HuMig: a new human member of the chemokine family of cytokines
JOURNAL    Biochem. Biophys. Res. Commun. 192 (1), 223-230 (1993)
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1981  tctcccatga  agaagggaag  cgggtgaagta  ctaagcgcta  gaggaagcag  ccaagtcggt
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2101  ggagggttcag  tgaattgtgt  aggagaggtt  gtctgtggcc  agaatttaaa  cctatactca
2161  ctttcccaaa  ttgaatcact  gctcacactg  ctgatgattt  agagtgcgtg  ccggtggaga
2221  tcccacccga  acgtcttctc  taatcatgaa  actccctagt  tccttcattg  aacttccctg
2281  aaaaatctaa  gtgtttcata  aatttgagag  tctgtgaccc  acttaccttg  catctcacag
2341  gtagacagta  tataactaac  aaccaagac  tacatattgt  cactgacaca  cacgttataa
2401  tcatttatca  tataataaca  tacatgcata  cactctcaaa  gcaaataatt  ttccattca
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LOCUS      AF002985      995 bp      mRNA      PRI      01-NOV-1997
DEFINITION Homo sapiens putative alpha chemokine (H174) mRNA, complete
            cds.
ACCESSION  AF002985
NID        g2580585
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 995)
AUTHORS    Jacobs,K.A., Collins-Racie,L.A., Colbert,M., Duckett,M.,
            Golden-Fleet,M., Kelleher,K., Kriz,R., LaVallie,E.R.,
            Merberg,D.,
            Spaulding,V., Stover,J., Williamson,M.J. and McCoy,J.M.
TITLE      A genetic selection for isolating cDNAs encoding secreted
            proteins
JOURNAL    Gene 198 (1-2), 289-296 (1997)
MEDLINE    98036061
REFERENCE  2 (bases 1 to 995)
AUTHORS    Jacobs,K.A., Collins-Racie,L.A., Colbert,M., Duckett,M.,
            Golden-Fleet,M., Kelleher,K., Kriz,R., LaVallie,E.R.,
            Merberg,D.,
            Spaulding,V., Stover,J., Williamson,M.J. and McCoy,J.M.
TITLE      Direct Submission
JOURNAL    Submitted (07-MAY-1997) Genetics Institute, 87 Cambridge Park
            Drive, Cambridge, MA 02140, USA
FEATURES   Location/Qualifiers
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181	tgcataggcc	ctggggtaaa	agcagtgaaa	gtggcagata
241	tacccaagta	acaactgtga	caaaatagaa	gtgattatta
301	caacgatgcc	taaatcccaa	atcgaagcaa	gcaaggctta
361	aagaattttt	aaaaatatca	aaacatatga	agtccctggaa
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661	catcggagtt	tacaaagtgc	tttcacgttc	ttacttgggtg
721	ctaggctaga	gaaccttcta	gatttgatgc	ttacaactat
781	atttctgtct	ctagaagtta	tctgtctgta	ttgatcttta
841	ttacagtggg	gacattgaca	ttattactgg	agtcaagccc
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LOCUS AF030514 1371 bp mRNA PRI 17-JUN-1998  
DEFINITION Homo sapiens interferon stimulated T-cell alpha chemoattractant precursor, mRNA, complete cds.  
ACCESSION AF030514  
NID g3219692  
KEYWORDS  
SOURCE human.  
ORGANISM Homo sapiens  
Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
REFERENCE 1 (bases 1 to 1371)  
AUTHORS Cole, K.E., Strick, C.A., Paradis, T.J., Ogborne, K.T., Loetscher, M., Gladue, R.P., Lin, W., Boyd, J.G., Moser, B., Wood, D.E., Sahagan, B.G. and Neote, K.  
TITLE Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3  
JOURNAL J. Exp. Med. 187 (12), 2009-2021 (1998)  
MEDLINE 98290735  
REFERENCE 2 (bases 1 to 1371)  
AUTHORS Cole, K.E., Strick, C.A. and Sahagan, B.G.  
TITLE Direct Submission  
JOURNAL Submitted (20-OCT-1997) Molecular Sciences, Pfizer, Inc., Eastern Point Road, Groton, CT 06340, USA  
FEATURES  
source Location/Qualifiers  
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/organism="Homo sapiens"  
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mat\_peptide 133..351  
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481 ttcaactttt gtacagttat gtgaaggatg aaaggtgggt gaaaggacca aaaacagaaa
541 tacagtcttc ctgaatgaat gacaatcaga attccactgc ccaaaggagt ccagcaatta
601 aatggatttc taggaaaagc taccttaaga aaggctgggt accatcgag tttacaaagt
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LOCUS      AF030514      1371 bp      mRNA      PRI      17-JUN-1998
DEFINITION Homo sapiens interferon stimulated T-cell alpha chemoattractant
            precursor, mRNA, complete cds.
ACCESSION  AF030514
NID        g3219692
KEYWORDS
SOURCE     human.
ORGANISM   Homo sapiens
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 1371)
AUTHORS    Cole,K.E., Strick,C.A., Paradis,T.J., Ogborne,K.T.,
Loetscher,M., Gladue,R.P., Lin,W., Boyd,J.G., Moser,B., Wood,D.E.,
Sahagan,B.G. and Neote,K.
TITLE      Interferon-inducible T cell alpha chemoattractant (I-TAC): a
novel      non-ELR CXC chemokine with potent activity on activated T cells
            through selective high affinity binding to CXCR3
JOURNAL     J. Exp. Med. 187 (12), 2009-2021 (1998)
MEDLINE     98290735
REFERENCE   2 (bases 1 to 1371)
AUTHORS     Cole,K.E., Strick,C.A. and Sahagan,B.G.
TITLE      Direct Submission
JOURNAL     Submitted (20-OCT-1997) Molecular Sciences, Pfizer, Inc.,
Eastern    Point Road, Groton, CT 06340, USA
FEATURES
            Location/Qualifiers
            source      1..1371
                        /organism="Homo sapiens"
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            sig_peptide 70..132
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mat_peptide 133..351
/evidence=not_experimental
/product="interferon stimulated T-cell alpha
chemoattractant"
BASE COUNT 487 a 228 c 244 g 411 t 1 others
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61 aaaacaaaca tgagtgtgaa gggcatggct atagccttgg ctgtgatatt gtgtgctaca
121 gttgttcaag gcttcccat gttcaaaaga ggacgctgtc tttgcatagg ccctggggta
181 aaagcagtga aagtggcaga tattgagaaa gcctccataa tgtaccaag taacaactgt
241 gacaaaatag aagtgttat taccctgaaa gaaaataaag gacaacgatg cctaaatccc
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LOCUS AF030514 1371 bp mRNA PRI 17-JUN-1998
DEFINITION Homo sapiens interferon stimulated T-cell alpha chemoattractant
precursor, mRNA, complete cds.
ACCESSION AF030514
NID g3219692
KEYWORDS
SOURCE human.
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1371)
AUTHORS Cole,K.E., Strick,C.A., Paradis,T.J., Ogborne,K.T.,
Loetscher,M., Gladue,R.P., Lin,W., Boyd,J.G., Moser,B., Wood,D.E.,
Sahagan,B.G. and Neote,K.
TITLE Interferon-inducible T cell alpha chemoattractant (I-TAC): a
novel non-ELR CXC chemokine with potent activity on activated T cells
through selective high affinity binding to CXCR3
JOURNAL J. Exp. Med. 187 (12), 2009-2021 (1998)
MEDLINE 98290735
REFERENCE 2 (bases 1 to 1371)
AUTHORS Cole,K.E., Strick,C.A. and Sahagan,B.G.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-1997) Molecular Sciences, Pfizer, Inc.,
Eastern Point Road, Groton, CT 06340, USA
FEATURES
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/db_xref="taxon:9606"
/chromosome="4"
/cell_type="astrocytes"
sig_peptide 70..132
CDS 70..354
/note="chemokine; I-TAC"
/codon_start=1

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mat\_peptide 133..351  
/evidence=not\_experimental  
/product="interferon stimulated T-cell alpha  
chemoattractant"

BASE COUNT 487 a 228 c 244 g 411 t 1 others  
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LOCUS HSMDNCF 1560 bp RNA PRI 31-MAR-1995  
DEFINITION Human mRNA for MDNCF (monocyte-derived neutrophil chemotactic factor).  
ACCESSION Y00787  
NID g34518  
KEYWORDS cytokine.  
SOURCE human.  
ORGANISM Homo sapiens  
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
REFERENCE 1 (bases 1 to 1560)  
AUTHORS Matsushima, K.  
TITLE Direct Submission  
JOURNAL Submitted (03-MAY-1988) Matsushima K., National Cancer Institute,, Bldg 560, Rm 31-19, Frederick, MD 21701  
REFERENCE 2 (bases 1 to 1560)  
AUTHORS Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Lew, W., Appella, E., Kung, H. F., Leonard, E. J. and Oppenheim, J. J.  
TITLE Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor  
JOURNAL J. Exp. Med. 167 (6), 1883-1893 (1988)  
MEDLINE 88258376  
COMMENT for overlapping sequence see M17016 - M17017.  
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241 aatttatcaa agaactgaga gtgattgaga gtggaccaca ctgcccaaac tgggtgcaga
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LOCUS HSINFGFR 1172 bp RNA PRI 21-MAR-1995
DEFINITION Human mRNA for gamma-interferon inducible early response gene
(with
homology to platelet proteins).
ACCESSION X02530 M17752
NID g33917
KEYWORDS interferon response; signal peptide.
SOURCE human.
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1172)
AUTHORS Luster,A.D., Unkeless,J.C. and Ravetch,J.V.
TITLE Gamma-interferon transcriptionally regulates an early-response
gene
containing homology to platelet proteins
JOURNAL Nature 315 (6021), 672-676 (1985)
MEDLINE 85240552
REFERENCE 2 (bases 1 to 1172)
AUTHORS Luster,A.D.
TITLE Direct Submission
JOURNAL Submitted (29-JUL-1986) to the EMBL/GenBank/DDBJ databases
COMMENT Data kindly reviewed (29-JUL-1986) by Luster A.D.
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BASE COUNT 384 a 231 c 208 g 349 t  
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121 attcaaggag tacctctctc tagaaccgta cgctgtacct gcatacagcat tagtaatcaa
181 cctgttaatc caaggtcttt agaaaaactt gaaattattc ctgcaagcca attttgtcca
241 cgtgttgaga tcattgtctac aatgaaaaag aagggtgaga agagatgtct gaatccagaa
301 tcgaaggcca tcaagaattt actgaaagca gttagcaagg aaatgtctaa aagatctcct
361 taaaaccaga ggggagcaaa atcgatgcag tgcttccaag gatggaccac acagaggctg
421 cctctcccat cacttcctca catggagtat atgtcaagcc ataattgttc ttagtttgca
481 gttactactaa aaggtgacca atgatggcca ccaaatcagc tgctactact cctgtaggaa
541 gggttaatggt catcatccta agctattcag taataactct accctggcac tataatgtaa
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721 tcagaatctc aaataactaa aagggtatgca atcaaatctg ctttttaaaag aatgctcttt
781 acttcatgga cttccactgc catcctccca aggggcccac attctttcag tggctaccta
841 catacaattc caaacacata caggaaggta gaaatatctg aaaatgtatg tgtaagtatt
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961 tttcagtgtg catggaataa catgtaatta agtactatgt atcaatgagt aacaggaaaa
1021 ttttaaaaat acagatagat atatgctctg catgttacat aagataaatg tgctgaatgg
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LOCUS SYNRP4A 225 bp DNA SYN 15-JUN-1989  
 DEFINITION Human recombinant platelet factor 4 (PF4) gene, complete cds.  
 ACCESSION M20901  
 NID g209285  
 KEYWORDS platelet factor; platelet factor 4.  
 SOURCE Synthetic oligonucleotide DNA, clone pIN-III-ompA-2.  
 ORGANISM artificial sequence  
 artificial sequence.  
 REFERENCE 1 (bases 1 to 225)  
 AUTHORS Barone,A.D., Ghrayeb,J., Hammerling,U., Zucker,M.B. and Thorbecke,G.J.  
 TITLE The expression in Escherichia coli of recombinant human platelet factor 4, a protein with immunoregulatory activity  
 JOURNAL J. Biol. Chem. 263, 8710-8715 (1988)  
 MEDLINE 88243725  
 FEATURES  
 source 1..225  
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 CDS <1..>225  
 /note="recombinant platelet factor 4"  
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BASE COUNT      59 a      59 c      51 g      56 t
ORIGIN          HindIII site.
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     61 tcagggttcgt ccgcgtcata tcacttctct ggaagttatc aaagctggtc cgcattgccc
    121 gactgctcag ctgatcgcta ctctcaaaaga cggtcgtaaa atctgcctgg acctgcaggg
    181 tccgctgtac aaaaaaatca tcaaaaaact gctggaatct ggatc
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LOCUS          HUMGRO          1050 bp      mRNA          PRI          11-JUN-1993
DEFINITION     Human gro (growth regulated) gene.
ACCESSION      J03561
NID            g183622
KEYWORDS       gro gene; tumor cell.
SOURCE         Human bladder tumor cell (T24) cDNA to mRNA.
ORGANISM       Homo sapiens
               Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
               Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE      1 (bases 1 to 1050)
AUTHORS        Anisowicz, A., Bardwell, L. and Sager, R.
TITLE          Constitutive overexpression of a growth-regulated gene in
               transformed Chinese hamster and human cells
JOURNAL        Proc. Natl. Acad. Sci. U.S.A. 84, 7188-7192 (1987)
MEDLINE        88041072
COMMENT        Draft entry and computer-readable sequence kindly submitted by
               R.Sager (20-NOV-1987).
FEATURES       Location/Qualifiers
               source          1..1050
               :              /organism="Homo sapiens"
               :              /db_xref="taxon:9606"
               sig_peptide    54..140
               :              /note="signal peptide (put.); putative"
               CDS            54..377
               :              /note="gro protein"
               :              /codon_start=1
               :              /db_xref="PID:g306806"

/translation="MARAALSAAPSNPRLRLVALLLLLLVAAGRRAAGASVATELRQC
CLQTLQGIHPKNIQSVNVKSPGPHCAQTEVIATLKNRKA CLNPASPIVKKII EKMLN
SDKSN"
mat_peptide    141..374
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BASE COUNT      270 a      246 c      239 g      295 t
ORIGIN          52 bp upstream of NcoI site.
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     61 gcgctgctct ctccgcgcgc cccagcaatc cccggctcct gcgagtggca ctgctgctcc
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    241 agtccccccg accccactgc gcccaaaccg aagtcatagc cacactcaag aatgggcgga
    301 aagcttgctt caatcctgca tccccatag ttaagaaaat catcgaaaag atgctgaaca
    361 gtgacaaatc caactgacca gaaggagga ggaagctcac tgggtgctgt tccatgaagga
    421 ggccctgccc ttataggaac agaagaggaa agagagacac agctgcagag gccacctgga
    481 ttgtgcctaa tgtgtttgag catcgcttag gagaagtctt ctatttattt atttattcat
    541 tagttttgaa gattctatgt taatatatta ggtgtaaaat aattaagggt atgattaact
    601 ctacctgcac actgtcctat tatattcatt ctttttgaaa tgtcaacccc aagttagttc
    661 aatctggatt catatttaat ttgaaggtag aatgttttca aatgttctcc agtcattatg
    721 ttaatatctt tgaggagcct gcaacatgcc agccactgtg atagaggctg gcggatccaa
    781 gcaaatggcc aatgagatca ttgtgaaggg aggggaatgt atgtgcacat ctgttttgta
    841 actgtttaga tgaatgtcag ttgttatatta ttgaaatgat ttcacagtgt gtggtcaaca
    901 tttctcatgt tgaaacttta agaactaaaa tgttctaata atcccttgga cattttatgt
    961 ctttcttgta aggcatactg cctgttttaa tggtagtttt acagtgtttc tggcttagaa
   1021 caaaggggct taattattga tgttttcgga
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LOCUS          HUMGROB5        1110 bp      mRNA          PRI          07-MAR-1995
DEFINITION     Human cytokine (GRO-beta) mRNA, complete cds.

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ACCESSION M36820  
 NID g183628  
 KEYWORDS cytokine.  
 SOURCE Human lymphocyte, cDNA to mRNA, clone GRO-beta.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 1110)  
 AUTHORS Haskill, S., Peace, A., Morris, J., Sporn, S.A., Anisowicz, A.,  
 Lee, S.W., Smith, T., Martin, G., Ralph, P. and Sager, R.  
 TITLE Identification of three related human GRO genes encoding  
 cytokine functions  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (19), 7732-7736 (1990)  
 MEDLINE 91017578  
 COMMENT Draft entry and computer-readable sequence for [Proc. Natl.  
 Acad. Sci. U.S.A. (1990) In press] kindly submitted  
 by S.Haskill, 20-JUL-1990.

FEATURES Location/Qualifiers  
 source 1..1110  
 /organism="Homo sapiens"  
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 gene 75..398  
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 CDS 75..398  
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/translation="MARATLSAAPSNPRLRLVALLLLLLVAASRRAGAPLATELRQC

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NGKSN"

BASE COUNT 300 a 247 c 247 g 316 t  
 ORIGIN

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121  tgcgggtggc gctgctgctc ctgctcctgg tggccgccag cgcgcgcgca gcaggagcgc
181  cccctggccac tgaactgcgc tgccagtgtc tgcagacctc gcagggaatt cacctcaaga
241  acatccaaag tgtgaagggtg aagtcctccg gaccccaactg cgcccaaacc gaagtcatag
301  ccacactcaa gaatgggcag aaagcttgct tcaaccccg ctcgcccag gttaaagaaa
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421  ttggtggctg ttcctgaagg aggccctgcc ttacaggaac agaagaggaa agagagacac
481  agctgcagag gccacctggc ttgcgcctaa tgtgtttgag catacttagg agaagcttc
541  tatttattta tttatttatt tatttggttg ttttagaaga ttctatgtta atattttatg
601  tgtaaaataa ggttatgatt gaactactct gcacactctc ccattatatt tattgtttat
661  tttaggtcaa acccaagtta gttcaatcct gattcatatt taatttgaag atagaagggt
721  tgcagatatt ctctagtcat ttgttaatat ttcttcgtga tgacatatca catgtcagcc
781  actgtgatag aggctgagga atccaagaaa atggccagta agatcaatgt gacgagcagg
841  aaatgtatgt gtgtctatct tgtaactgta aagatgaatg tcagttgtta tttattgaaa
901  tgatttcaca gtgtgtggtc aacatttctc atgttgaagc tttagaactt aaaaatgtct
961  aaatatccct tggcatttta tgtcttctct gtaagatact gccttggtta atgttaatta
1021  tgcagtgttt cctctgtgtg tagagcagag aggtttcgat atttattgat gttttcacia
1081  agaacaggaa aataaaatat ttaaaatat
  
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LOCUS HUMGROG5 1064 bp mRNA PRI 07-MAR-1995  
 DEFINITION Human cytokine (GRO-gamma) mRNA, complete cds.  
 ACCESSION M36821  
 NID g183632  
 KEYWORDS cytokine.  
 SOURCE Human lymphocyte, cDNA to mRNA, clone GRO-gamma.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 1064)  
 AUTHORS Haskill, S., Peace, A., Morris, J., Sporn, S.A., Anisowicz, A.,



TITLE Lee, S.W., Smith, T., Martin, G., Ralph, P. and Sager, R.  
 cytokine Identification of three related human GRO genes encoding  
 functions  
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (19), 7732-7736 (1990)  
 MEDLINE 91017578  
 COMMENT Draft entry and computer-readable sequence for [Proc. Natl.  
 Acad. Sci. U.S.A. (1990) In press] kindly submitted  
 by S.Haskill, 20-JUL-1990.  
 FEATURES Location/Qualifiers  
 source 1..1064  
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 CDS 78..398  
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 GSTN"  
 BASE COUNT 281 a 237 c 239 g 305 t 2 others  
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 61 cgcgtctgct gagccccatg gcccacgccca cgctctccgc cgccccagc aatccccggc  
 121 tcctgcggtt ggcgctgctg ctccctgctc tgggtggcag ccggcgcgca gcaggagcgt  
 181 ccgtgggtcac tgaactgcgc tgccagtgc tgcagacact gcagggaatt caccctcaaga  
 241 acatccaaag tgtgaatgta aggtccccc gacccactg cgcccaaacc gaagtcatag  
 301 ccacactcaa gaatgggaag aaagcttgct tcaacccgc atcccccatg gttcagaaaa  
 361 tcatcgaaaa gatactgaac aaggggagca ccaactgaca ggagagaagt aagaagctta  
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 541 cttacgaggg ttctacttat ttatgtatct atttttgaaa gcttgtatct taatatttta  
 601 catgctgtta tttaaagatg tgagtgtggt tcatcaaaaca tagctcagtc ctgattatct  
 661 aattggaata tgatgggttt taaatgtgtc attaaactaa tattagtgg gagaccataa  
 721 tgtgtcagcc accttgataa atgacagggt ggggaactgg agggtngggg gattgaaatg  
 781 caagcaatta gtggatcact gttagggtaa gggaaatgat gtacacatct attttttata  
 841 cttttttttt taaaaaagaa tgtcagttgt tattttattca aattatctca cattatgtgt  
 901 tcaacatttt tatgctgaag ttcccttag acattttatg tcttgcctgt agggcataat  
 961 gccttggtta atgtccattc tgcagcgttt ctctttccct tggaaaagag aatttatcat  
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LOCUS HUMCTAP3 673 bp mRNA PRI 06-MAR-1995  
 DEFINITION Human connective tissue activation peptide III mRNA, complete  
 cds.  
 ACCESSION M54995 M38441  
 NID g181175  
 KEYWORDS connective tissue activating peptide-III; platelet basic  
 protein;  
 thromboglobulin.  
 SOURCE Human platelet, cDNA to mRNA, clone lambda-c[1,2].  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 673)  
 AUTHORS Wenger, R.H., Wicki, A.N., Walz, A., Kieffer, N. and Clemetson, K.J.  
 TITLE Cloning of cDNA coding for connective tissue activating peptide  
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 from a human platelet-derived lambda gt11 expression library  
 JOURNAL Blood 73 (6), 1498-1503 (1989)  
 MEDLINE 89229374  
 FEATURES Location/Qualifiers  
 source 1..673

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mat_peptide 208..450
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61 tccaccatga gcctcagact tgataccacc ccttcctgta acagtgcgag accacttcat
121 gccttgcagg tgctgctgct tctgtcattg ctgctgactg ctctggcttc ctccaccaa
181 ggacaaacta agagaaactt ggcgaaaggc aaagaggaaa gtctagacag tgacttggat
241 gctgaactcc gctgcatgtg tataaagaca acctctggaa ttcattccaa aaacatccaa
301 agtttggaag tgatcgggaa aggaacccat tgcaaccaag tcgaagtgat agccacactg
361 aaggatggga ggaaaatctg cctggaccca gatgctccca gaatcaagaa aattgtacag
421 aaaaaattgg caggtgatga atctgctgat taattgttc tgtttctgcc aaacttcttt
481 aactcccagg aagggttaga ttttgaaacc ttgattttct agagtcttca tttattcagg
541 atacctattc ttactgtatt aaaatttgga tatgtgttct attctgtctc aaaaatcaca
601 ttttattctg agaaggttgg taaaagatg gcagaaagaa gatgaaata aataagcctg
661 gtttcaacct tct
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LOCUS HUMENA78A 2177 bp DNA PRI 31-JAN-1996
DEFINITION Homo sapiens neutrophil-activating peptide 78 (ENA-78) gene,
complete cds.
ACCESSION L37036 Z46254
NID g607030
KEYWORDS ENA-78 gene; homologue; neutrophil-activating factor;
neutrophil-activating peptide 78.
SOURCE Homo sapiens DNA.
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 2177)
AUTHORS Walz,A., Burgener,R., Car,B., Baggiolini,M., Kunkel,S.L. and
Strieter,R.M.
TITLE Structure and neutrophil-activating properties of a novel
inflammatory peptide (ENA-78) with homology to interleukin 8
J. Exp. Med. 174 (6), 1355-1362 (1991)
MEDLINE 92078844
REFERENCE 2 (bases 1 to 2177)
AUTHORS Walz,A.

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TITLE Direct Submission  
 JOURNAL Submitted (14-OCT-1994) A. Walz, University of Bern, Theodor  
 Kocher  
 Institute, Freiestr. 1, Bern, Switzerland 3012  
 REFERENCE 3 (bases 1 to 2177)  
 AUTHORS Corbett, M.S., Schmitt, I., Riess, O. and Walz, A.  
 TITLE Characterization of the gene for human neutrophil-activating  
 peptide 78 (ENA-78)  
 JOURNAL Biochem. Biophys. Res. Commun. 205 (1), 612-617 (1994)  
 MEDLINE 95091791

FEATURES  
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 CDS join(803..911,1046..1178,1289..1372,1729..1747)  
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 912..1045  
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 intron 1373..1728  
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 exon 1729..>1747  
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BASE COUNT 539 a 512 c 496 g 630 t  
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121 tatcttggtt tccaaagctg gttgaagtcc agagattcat aaagtcattc aagaaacctt
181 gaatgacctg cctgcaagaa gacaggaagg actttcagtt tatagcaatt caaacatgaa
241 taacatttcc tgattaatag taataataat tagaaaggat tgactttcag aaatttttct
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421 atgaggcgct gctgaatcct gctgaatagc tactcccttc tagctggagc cacagctccc
481 tccaccgcgg aacagggtta caacgtccct ctcggtagag gtgcacgcag ctccctcttg
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601 tgcccatgaa gtccagggaa ttccccagc atcccaaagc ttgagtttcc tgtcagtggt
661 gagagatgag tgtagataaa aggagtgcag aaggaacgag gaagccacag tgctccggat
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LOCUS HSGCP2 254 bp RNA PRI 04-MAR-1997

DEFINITION H. sapiens mRNA for granulocyte chemotactic protein.

ACCESSION Y08770

NID g1769436

KEYWORDS cell surface receptor; CXC chemokine; GCP-2 gene; granulocyte chemotactic protein.

SOURCE human.

ORGANISM Homo sapiens

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 254)

AUTHORS Froyen, G., Proost, P., Ronsse, I., Mitera, T., Haelens, A., Wuyts, A., Opdenakker, G., Van Damme, J. and Billiau, A.

TITLE Cloning, bacterial expression and biological characterization of recombinant human granulocyte chemotactic protein-2 and differential expression of granulocyte chemotactic protein-2 and epithelial cell-derived neutrophil activating peptide-78 mRNAs

JOURNAL Eur. J. Biochem. 243 (3), 762-769 (1997)

MEDLINE 97210779

REFERENCE 2 (bases 1 to 254)

AUTHORS Froyen, G.F.V.

TITLE Direct Submission

JOURNAL Submitted (10-OCT-1996) G.F.V. Froyen, Rega Institute, University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, BELGIUM

FEATURES

Location/Qualifiers

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NID        g1754608
KEYWORDS   SCM-1beta; SCM-1beta precursor.
SOURCE     Homo sapiens placenta DNA, clone:hg44.
ORGANISM   Homo sapiens
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            Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
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            Homo.
REFERENCE  1 (sites)
AUTHORS    Yoshida,T., Imai,T., Kakizaki,M., Nishimura,M. and Yoshie,O.
TITLE      Molecular cloning of a novel C or gamma type chemokine, SCM-1
JOURNAL    FEBS Lett. 360 (2), 155-159 (1995)
MEDLINE    95180438
REFERENCE  2 (sites)
AUTHORS    Yoshida,T., Imai,T., Takagi,S., Nishimura,M., Ishikawa,I.,
Yaoi,T.    and Yoshie,O.
TITLE      Structure and expression of two highly related genes encoding
            SCM-1/human lymphotactin
JOURNAL    FEBS Lett. 395 (1), 82-88 (1996)
MEDLINE    97002294
REFERENCE  3 (bases 1 to 5669)
AUTHORS    Yoshida,T.
JOURNAL    Unpublished (1995)
REFERENCE  4 (bases 1 to 5669)
AUTHORS    Yoshida,T.
TITLE      Direct Submission
JOURNAL    Submitted (07-AUG-1995) to the DDBJ/EMBL/GenBank databases.
Tetsuya    Yoshida, Shionogi Institute for Medical Science; 2-5-1,
Mishima,   Settsu, Osaka 566, Japan (E-
mail:teyoshid@fl.lab.shionogi.co.jp,
Tel:06-382-2612, Fax:06-382-2598)
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DEFINITION Human DNA for SCM-lalpha precursor, complete cds.
ACCESSION  D63790
NID        g1754610
KEYWORDS   SCM-lalpha precursor; SCM-1 alpha.
SOURCE     Homo sapiens placenta DNA, clone:hg40.
ORGANISM   Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE  1 (sites)
AUTHORS    Yoshida,T., Imai,T., Kakizaki,M., Nishimura,M. and Yoshie,O.
TITLE      Molecular cloning of a novel C or gamma type chemokine, SCM-1
JOURNAL    FEBS Lett. 360 (2), 155-159 (1995)

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MEDLINE 95180438  
 REFERENCE 2 (sites)  
 AUTHORS Yoshida,T., Imai,T., Takagi,S., Nishimura,M., Ishikawa,I.,  
 Yaoi,T.  
 TITLE and Yoshie,O.  
 Structure and expression of two highly related genes encoding  
 SCM-1/human lymphotactin  
 JOURNAL FEBS Lett. 395 (1), 82-88 (1996)  
 MEDLINE 97002294  
 REFERENCE 3 (bases 1 to 5660)  
 AUTHORS Yoshida,T.  
 JOURNAL Unpublished (1995)  
 REFERENCE 4 (bases 1 to 5660)  
 AUTHORS Yoshida,T.  
 TITLE Direct Submission  
 JOURNAL Submitted (07-AUG-1995) to the DDBJ/EMBL/GenBank databases.  
 Tetsuya  
 Yoshida, Shionogi Institute for Medical Science; 2-5-1,  
 Mishima, Settsu, Osaka 566, Japan (E-  
 mail:teyoshid@f1.lab.shionogi.co.jp,  
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AUTHORS      Bazan,J.F., Bacon,K.B., Hardiman,G., Wang,W., Soo,K., Rossi,D.,
              Greaves,D.R., Zlotnik,A. and Schall,T.J.
TITLE        A new class of membrane-bound chemokine with a CX3C motif
JOURNAL      Nature 385 (6617), 640-644 (1997)
MEDLINE      97177111
REFERENCE    2 (bases 1 to 1635)
AUTHORS      Bazan,J.F., Bacon,K.B., Hardiman,G., Wang,W., Rossi,D.,
              Greaves,D.R., Zlotnik,A. and Schall,T.J.
TITLE        Direct Submission
JOURNAL      Submitted (03-MAR-1997) Molecular Biology, DNAX Research
              Institute,
              901 California Ave., Palo Alto, CA 94304-1104, USA
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 DEFINITION Human CX3C chemokine precursor, mRNA, alternatively spliced,  
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 ACCESSION U84487  
 NID g1888522  
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 SOURCE human.  
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 REFERENCE 1 (bases 1 to 3310)  
 AUTHORS Bazan, J.F., Bacon, K.B., Hardiman, G., Wang, W., Soo, K., Rossi, D.,  
 Greaves, D.R., Zlotnik, A. and Schall, T.J.  
 TITLE A new class of membrane-bound chemokine with a CX3C motif  
 JOURNAL Nature 385 (6617), 640-644 (1997)  
 MEDLINE 97177111  
 REFERENCE 2 (bases 1 to 3310)  
 AUTHORS Bazan, J.F., Bacon, K.B., Hardiman, G., Wang, W., Rossi, D.,  
 Greaves, D.R., Zlotnik, A. and Schall, T.J.  
 TITLE Direct Submission  
 JOURNAL Submitted (07-JAN-1997) Molecular Biology, DNAX Research  
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ACCESSION  U91746
NID        g2581780
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REFERENCE  1 (bases 1 to 1430)
AUTHORS    Hedrick,J.A., Helms,A., Gorman,D. and Zlotnik,A.
TITLE      Identification of a novel human CC chemokine upregulated by IL-
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JOURNAL    Blood (1998) In press
REFERENCE  2 (bases 1 to 1430)
AUTHORS    Hedrick,J.A., Helms,A., Gorman,D. and Zlotnik,A.
TITLE      Direct Submission
JOURNAL    Submitted (02-MAR-1997) Immunology, DNAX Research Institute,
901        California Ave, Palo Alto, CA 94304, USA
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/26291

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(b) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/84, 85.1, 184.1, 186.1, 188.1, 278.1; 514/2, 8, 12, 44; 530/300, 324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,141, 867 A (IVANOFF et al.) 25 August 1992, see entire document.	22-32, 45-55
A	ENG et al. The Stimulatory Effects of Interleukin (IL)-12 On Hematopoiesis Are Antagonized by IL-12-induced Interferon $\gamma$ In Vivo. J. Exp. Med. May 1995, Vol.181, pages 1893-1898, see entire document.	1-21, 33-44
A	ORANGE et al. Mechanism of Interleukin 12-mediated Toxicities during Experimental Viral Infections: Role of Tumor Necrosis Factor and Glucocorticoids. J. Exp. Med. March 1995, Vol.181, pages 901-914, see entire document.	1-21, 33-44

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 MARCH 1999

Date of mailing of the international search report

15 APR 1999

Name and mailing address of the ISA/US  
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/26291

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WU et al. Receptor-mediated in Vitro Gene Transformation by a Soluble DNA Carrier System. The Journal of Biological Chemistry. 05 April 1987, Vol.252, No. 10, pages 4429-4432, see entire document.	22-32, 45-55



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/26291

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/26291

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07K 14/47, 14/52; C12N 15/12, 15/19, 15/63; A61K 38/16, 38/19, 48/00

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/84, 85.1, 184.1, 186.1, 188.1, 278.1; 514/2, 8, 12, 44; 530/300, 324

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAN ONLINE, MEDLINE, CAPLUS

search terms: chemokine, vaccination, immunogenic, antigen, HIV, efficacy, macrophage-derived chemokine, stromal cell-derived factor, monocyte chemotactic protein, composition, administration

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claims 1-21, 33-44, drawn to a method to enhance the efficacy of a vaccine in a subject comprising administering an antigen and one or more chemokines and a composition thereto.

Group II, claims 22-32, 45-55, drawn to a method to enhance the efficacy of a vaccine in a subject comprising administering nucleic acid sequences encoding one or more antigens and nucleic acid sequences encoding one or more chemokines.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product and method, a method to enhance the efficacy of a vaccine in a subject comprising administering an antigen and one or more chemokines and a composition thereto. Further pursuant to 37

C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.